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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup>:</b> <b>C12N 15/57, 9/54, C11D 3/386</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 95/30011</b> <b>(43) International Publication Date:</b> 9 November 1995 (09.11.95)
<b>(21) International Application Number:</b> PCT/US95/04760 <b>(22) International Filing Date:</b> 18 April 1995 (18.04.95) <b>(30) Priority Data:</b> 08/237,938 2 May 1994 (02.05.94) US 08/400,068 7 March 1995 (07.03.95) US <b>(71) Applicant:</b> THE PROCTER & GAMBLE COMPANY [US/US]; One Procter & Gamble Plaza, Cincinnati, OH 45202 (US). <b>(72) Inventors:</b> BRODE, Philip, Frederick, III; 5780 Squirrelsneet Lane, Cincinnati, OH 45252 (US). BARNETT, Bobby, Lee; 12175 Elkwood Drive, Cincinnati, OH 45240 (US). RUBINGH, Donn, Nelton; 8224 Sheed Road, Cincinnati, OH 45247 (US). GHOSH, Chanchal, Kamur; 7005 Pinemill Drive, West Chester, OH 45069 (US). <b>(74) Agents:</b> REED, T., David et al.; The Procter & Gamble Company, 5299 Spring Grove Avenue, Cincinnati, OH 45217 (US).	<b>(81) Designated States:</b> AM, AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>	
<b>(54) Title:</b> SUBTILISIN 309 VARIANTS HAVING DECREASED ADSORPTION AND INCREASED HYDROLYSIS  <b>(57) Abstract</b> <p>The present invention relates to subtilisin (309) variants having a modified amino acid sequence of wild-type subtilisin (309) amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region, a fifth loop region and a sixth loop region; wherein the modified amino acid sequence comprises different amino acids than that occurring in wild-type subtilisin (309) (i.e., substitution) at specifically identified positions in one or more of the loop regions whereby the subtilisin (309) variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild type subtilisin (309). The present invention also relates to DNA sequence encoding such subtilisin (309) variants. The present invention also relates to compositions comprising such subtilisin (309) variants for cleaning a variety of surfaces.</p>		

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## SUBTILISIN 309 VARIANTS HAVING DECREASED ADSORPTION AND INCREASED HYDROLYSIS

### TECHNICAL FIELD

5       The present invention relates to novel enzyme variants useful in a variety of cleaning compositions, and the genes encoding such enzyme variants.

### BACKGROUND

10       Enzymes make up the largest class of naturally occurring proteins. Each class of enzyme generally catalyzes (accelerates a reaction without being consumed) a different kind of chemical reaction. One class of enzymes known as proteases, are known for their ability to hydrolyze (break down a compound into two or more simpler compounds with the uptake of the H and OH parts of a water molecule on either side of the chemical bond cleaved)

15       other proteins. This ability to hydrolyze proteins has been taken advantage of by incorporating naturally occurring and protein engineered proteases as an additive to laundry detergent preparations. Many stains on clothes are proteinaceous and wide-specificity proteases can substantially improve removal of such stains.

20       Unfortunately, the efficacy level of these proteins in their natural, bacterial environment, frequently does not translate into the relatively unnatural wash environment. Specifically, protease characteristics such as thermal stability, pH stability, oxidative stability and substrate specificity are not necessarily optimized for utilization outside the natural environment of the

25       enzyme.

30       The amino acid sequence of the protease determines the characteristics of the protease. A change of the amino acid sequence of the protease may alter the properties of the enzyme to varying degrees, or may even inactivate the enzyme, depending upon the location, nature and/or magnitude of the change in the amino acid sequence. Several approaches have been taken to alter the wild-type amino acid sequence of proteases in an attempt to improve their properties, with the goal of increasing the efficacy of the protease in the wash environment. These approaches include altering the amino acid sequence to enhance thermal stability and to improve oxidation

35       stability under quite diverse conditions.

      Despite the variety of approaches described in the art, there is a continuing need for new effective variants of proteases useful for cleaning a variety of surfaces.

### Objects of the Present Invention

It is an object of the present invention to provide subtilisin 309 enzyme variants having improved hydrolysis versus the wild-type of the enzyme.

It is also an object of the present invention to provide cleaning compositions comprising these subtilisin 309 enzyme variants.

### SUMMARY

The present invention relates to subtilisin 309 variants having a modified amino acid sequence of wild-type subtilisin 309 amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region, a fifth loop region and a sixth loop region; wherein the modified amino acid sequence comprises different amino acids than that occurring in wild-type subtilisin 309 (i.e., substitution) at specifically identified positions in one or more of the loop regions whereby the subtilisin 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type subtilisin 309. The present invention also relates to DNA sequences encoding such subtilisin 309 variants. The present invention also relates to compositions comprising such subtilisin 309 variants for cleaning a variety of surfaces.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A and Figure 1B depict the amino acid sequences of subtilisin BPN' and subtilisin 309. The top line represents the amino acid sequence of subtilisin BPN' (SEQ ID NO:2), which is derived from *Bacillus amyloliquefaciens*; the bottom line represents the amino acid sequence of subtilisin 309 (SEQ ID NO:1), which is derived from *Bacillus lentus*. The symbol \* in the sequence for subtilisin 309 denotes the absence of specific amino acid residues as compared to subtilisin BPN'.

### DESCRIPTION

#### I. Subtilisin 309 Variants

This invention pertains to subtilisin enzymes, in particular subtilisin 309, that have been modified by mutating the various nucleotide sequences that code for the enzyme, thereby modifying the amino acid sequence of the enzyme. The modified subtilisin enzymes (hereinafter, "subtilisin 309 variants") of the present invention have decreased adsorption to and increased hydrolysis of an insoluble substrate as compared to the wild-type subtilisin. The present invention also pertains to the mutant genes encoding for such subtilisin 309 variants.

When referring to the amino acid sequence of native subtilisin 309



(having 269 amino acid residues), the amino acid sequence of subtilisin BPN' (having 275 amino acid residues) is frequently used as the standard. The use of "BPN' numbering" has become the conventional method for identifying residue positions in all subtilisins. The amino acid sequences for native subtilisin BPN' and native subtilisin 309 are set forth in Figs 1A and 1B. The symbol '\*' in the sequence for subtilisin 309 in Figs 1A and 1B denotes the absence of specific amino acid residues compared to native subtilisin BPN'. However, for purposes of discussion herein, reference to amino acid positions shall be based on "true" subtilisin 309 numbering (e.g., refer to SEQ ID NO:1, discussed below).

The subtilisin 309 enzymes of this invention belong to a class of enzymes known as proteases. A protease is a catalyst for the cleavage of peptide bonds. One type of protease is a serine protease. A serine protease is distinguished by the fact that there is an essential serine residue at the active site.

The observation that an enzyme's rate of hydrolysis of soluble substrates increases with enzyme concentration is well documented. It would therefore seem plausible that for surface bound substrates, such as is encountered in many cleaning applications, the rate of hydrolysis would increase with increasing surface concentration. This has been shown to be the case. (Brode, P.F. III and D. S. Rauch, LANGMUIR, "Subtilisin BPN': Activity on an Immobilized Substrate", Vol. 8, pp. 1325-1329 (1992)). In fact, a linear dependence of rate upon surface concentration was found for insoluble substrates when the surface concentration of the enzyme was varied. (Rubingh, D. N. and M. D. Bauer, "Catalysis of Hydrolysis by Proteases at the Protein-Solution Interface," in POLYMER SOLUTIONS, BLENDS AND INTERFACES, Ed. by I. Noda and D. N. Rubingh, Elsevier, p. 464 (1992)). Surprisingly, when seeking to apply this principle in the search for variant proteases which give better cleaning performance, we did not find that enzymes which adsorb more give better performance. In fact, we surprisingly determined the opposite to be the case: decreased adsorption by an enzyme to a substrate resulted in increased hydrolysis of the substrate (i.e., better cleaning performance).

While not wishing to be bound by theory, it is believed that improved performance, when comparing one variant to another, is a result of the fact that enzymes which adsorb less are also less tightly bound and therefore more highly mobile on the surface from which the insoluble protein substrate is to be removed. At comparable enzyme solution concentrations, this increased mobility is sufficient to outweigh any advantage that is conferred by delivering

a higher concentration of enzyme to the surface.

The mutations described herein are designed to change (i.e., decrease) the adsorption of the enzyme to surface-bound soils. In subtilisin 309, certain amino acids form exterior loops on the enzyme molecule. For purposes of discussion, these loops shall be referred to as the first, second, third, fourth and fifth loop regions. Specifically, positions 57-64 form the first loop region; positions 93-105 form the second loop region; positions 124-131 form the third loop region; positions 152-161 form the fourth loop region; positions 181-185 form the fifth loop region; and positions 193-214 form the sixth loop region (position numbering analagous to positions in the amino acid sequence for wild-type subtilisin 309 (SEQ ID NO:1)).

It is believed that these loop regions play a significant role in the adsorption of the enzyme molecule to a surface-bound peptide, and specific mutations in one or more of these loop regions will have a significant effect on this adsorption. While not wishing to be bound by theory, it is believed that the loop regions are important to the adsorption of the subtilisin 309 molecule for at least two reasons. First, the amino acids which comprise the loop regions can make close contacts with any surfaces to which the molecule is exposed. Second, the proximity of the loop regions to the active-site and binding pocket of the subtilisin 309 molecule gives them a role in the catalytically productive adsorption of the enzyme to surface-bound substrates (peptides/protein soils).

The following is a list of abbreviations used herein to describe amino acids:

	Amino Acid	Three-letter Abbreviation	One-letter Symbol
	Alanine	Ala	A
	Arginine	Arg	R
	Asparagine	Asn	N
5	Aspartic Acid	Asp	D
	Asparagine or Aspartic Acid	Asp	D
	Cysteine	Cys	C
	Glutamine	Gln	Q
	Glutamic Acid	Glu	E
10	Glutamine or Glutamic Acid	Glx	Z
	Glycine	Gly	G
	Histidine	His	H
	Isoleucine	Ile	I
	Leucine	Leu	L
15	Lysine	Lys	K
	Methionine	Met	M
	Phenylalanine	Phe	F
	Proline	Phe	F
	Serine	Ser	S
20	Threonine	Thr	T
	Tryptophan	Trp	W
	Tyrosine	Tyr	Y
	Valine	Val	V
	No amino acid at position	Xaa	*

25 As used herein, "variant" means an enzyme having an amino acid sequence which differs from that of wild-type.

As used herein, "mutant subtilisin 309 DNA sequence" means a DNA sequence coding for a subtilisin 309 variant.

30 As used herein, "wild-type subtilisin 309" refers to an enzyme represented by SEQ ID NO:1. The amino acid sequence for subtilisin 309 is further described in World Patent Publication 89/06279 (1989), incorporated herein by reference. See also, World Patent Publication 94/02618, published February 3, 1994 by Mulleners et al.

35 As used herein, the term "subtilisin 309 wild-type amino acid sequence" encompasses SEQ ID NO:1 as well as SEQ ID NO:1 having modifications to the amino acid sequence other than at any of positions 57-64, 93-105, 124-131, 152-161, 181-185 and 193-214.

As used herein, "more hydrophilic amino acid" refers to any other amino

acid having greater hydrophilicity than a subject amino acid with reference to the hydrophilicity table below. The following hydrophilicity table (Table 1) lists amino acids in descending order of increasing hydrophilicity (see Hopp, T.P., and Woods, K.R., "Prediction of Protein Antigenic Determinants from Amino Acid Sequences", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE USA, Vol. 78, pp. 3824-3828, 1981, incorporated herein by reference).

TABLE 1

	Amino Acid	Hydrophilicity Value
	Trp	-3.4
10	Phe	-2.5
	Tyr	-2.3
	Leu, Ile	-1.8
	Val	-1.5
	Met	-1.3
15	Cys	-1.0
	Ala, His	-0.5
	Thr	-0.4
	Pro, Gly	-0.0
	Gln, Asn	0.2
20	Ser	0.3
	Arg <sup>+</sup> , Lys <sup>+</sup> , Glu <sup>-</sup> , Asp <sup>-</sup>	3.0

Table 1 also indicates which amino acids carry a charge (this characteristic being based on a pH of from about 8-9). The positively charged amino acids are Arg and Lys, the negatively charged amino acids are Glu and Asp, and the remaining amino acids are neutral. In a preferred embodiment of the present invention, the substituting amino acid is either neutral or negatively charged, more preferably negatively charged (i.e., Glu or Asp).

Therefore, for example, the statement "substitute Gln with an equally or more hydrophilic amino acid which is neutral or has a negative charge" means Gln would be substituted with Asn (which is equally hydrophilic to Gln), or Ser, Glu or Asp (which are more hydrophilic than Gln); each of which are neutral or have a negative charge, and have a greater hydrophilicity value as compared to Gln. Likewise, the statement "substitute Pro with a more hydrophilic amino acid which is neutral or has a negative charge" means Pro would be substituted with Gln, Asn, Ser, Glu or Asp.

A. Loop Region 6 Substitution Variants1. Variants comprising at least one amino acid substitution

In one embodiment of the present invention, the subtilisin 309 variant has a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, wherein the modified amino acid sequence comprises a substitution at one or more of positions 193, 194, 195, 196, 197, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214; whereby the subtilisin 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type subtilisin 309. Preferably, the positions having a substituted amino acid are 193, 194, 195, 196, 199, 201, 202, 203, 204, 205, 206 or 209; more preferably, 194, 195, 196, 199 or 201.

Preferably, the substituting amino acid for position 193 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Pro, Ser or Thr.

Preferably, the substituting amino acid for position 194 is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

Preferably, the substituting amino acid for position 195 is Asn, Asp, Gln, Glu, Gly, Ser.

Preferably, the substituting amino acid for position 196 is Asn, Asp, Gln, Glu, Pro or Ser.

Preferably, the substituting amino acid for position 197 is Ala, Asp, Cys, Gln, Glu, Gly, His, Met, Pro or Ser.

Preferably, the substituting amino acid for position 199 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

Preferably, the substituting amino acid for position 200 is Asn, Asp, Glu or Ser.

Preferably, the substituting amino acid for position 201 is Asp or Glu.

Preferably, the substituting amino acid for position 202 is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

Preferably, the substituting amino acid for position 203 is Ala, Asn, Asp, Gln, His, Ile, Met, Pro or Ser.

Preferably, the substituting amino acid for position 204 is Asn, Asp, Gln, Glu, Gly or Ser.

Preferably, the substituting amino acid for position 205 is Asn, Asp, Gln, Glu, Pro or Ser.

Preferably, the substituting amino acid for position 206 is Asp or Glu.

Preferably, the substituting amino acid for position 207 is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

Preferably, the substituting amino acid for position 208 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro or Val.

Preferably, the substituting amino acid for position 209 is Asn, Asp, Gln, Glu, gly, His, Pro, Ser or Thr.

5 Preferably, the substituting amino acid for position 210 is Asp or Glu.

Preferably, the substituting amino acid for position 211 is Ala, Asn, Asp, Cys, Gln, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

Preferably, the substituting amino acid for position 212 is Glu.

10 Preferably, the substituting amino acid for position 213 is Asn, Asp, Gln, Glu, Pro or Ser.

Preferably, the substituting amino acid for position 214 is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

15 More preferably, the substituting amino acid for any of positions 193, 194, 195, 196, 197, 199, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 and 214 is, with reference to Table 1, is neutral or negatively charged and equally or more hydrophilic, preferably more hydrophilic, than the amino acid at the subject position in wild-type subtilisin 309.

20 More preferably still, the substituting amino acid for any of positions 193, 194, 195, 196, 197, 199, 200, 201, 202, 204, 205, 206, 207, 208, 209, 210, 212, 213 and 214 is Asp or Glu; and the substituting amino acid for positions 203 and 211 is Asp.

## 2. Variants comprising at least two amino acid substitutions

25 In another embodiment of the present invention, the subtilisin 309 variant has a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, wherein the modified amino acid sequence comprises a substitution at two or more of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214; whereby the subtilisin 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309.

30 Preferably, the positions having a substituting amino acid are 193, 194, 195, 199, 201, 202, 203, 204, 205, 206, or 209; more preferably, positions 194, 195, 196, 211 or 213.

Preferably, the substituting amino acid for position 193 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser and Thr.

35 Preferably, the substituting amino acid for position 194 is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

Preferably, the substituting amino acid for position 195 is Asn, Asp, Gln, Glu, Gly or Ser.

Preferably, the substituting amino acid for position 196 is Asn, Asp, Gln, Glu, Pro or Ser.

Preferably, the substituting amino acid for position 197 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

5 Preferably, the substituting amino acid for position 198 is Asp, Gln, Glu or Ser.

Preferably, the substituting amino acid for position 199 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

10 Preferably, the substituting amino acid for position 200 is Asn, Asp, Glu or Ser.

Preferably, the substituting amino acid for position 201 is Asp or Glu.

Preferably, the substituting amino acid for position 202 is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

15 Preferably, the substituting amino acid for position 203 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser or Thr.

Preferably, the substituting amino acid for position 204 is Asn, Asp, Gln, Glu, Gly or Ser.

Preferably, the substituting amino acid for position 205 is Asn, Asp, Gln, Glu, Pro or Ser.

20 Preferably, the substituting amino acid for position 206 is Asp or Glu.

Preferably, the substituting amino acid for position 207 is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

Preferably, the substituting amino acid for position 208 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro or Val.

25 Preferably, the substituting amino acid for position 209 is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

Preferably, the substituting amino acid for position 210 is Asp or Glu.

Preferably, the substituting amino acid for position 211 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

30 Preferably, the substituting amino acid for position 212 is Asp, Gln, Glu or Ser. However, if position 211 is substituted with Asn, Glu or Val, then position 212 is not substituted with Asp;

Preferably, the substituting amino acid for position 213 is Asn, Asp, Gln, Glu, Pro or Ser.

35 Preferably, the substituting amino acid for position 214 is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

More preferably, the substituting amino acid for any of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208,

209, 210, 211, 212, 213 or 214 is, with reference to Table 1, is neutral or negatively charged and equally or more more hydrophilic, preferably more hydrophilic, than the amino acid at the subject position in wild-type subtilisin 309.

- 5 More preferably still, the substituting amino acid for any of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 is Asp and Glu.

3. Variants comprising at least three amino acid substitutions

- 10 In another embodiment of the present invention, the subtilisin 309 variant has a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, wherein the modified amino acid sequence comprises a substitution at three or more of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214; whereby the subtilisin 309 variant has decreased adsorption to, and increased  
15 hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309. Preferably, the positions having a substituting amino acid are 193, 194, 195, 199, 201, 202, 203, 204, 205, 206, or 209; more preferably, positions 194, 195, 196, 211 or 213.

- 20 Preferably, the substituting amino acid for position 193 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

Preferably, the substituting amino acid for position 194 is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

Preferably, the substituting amino acid for position 195 is Asn, Asp, Gln, Glu, Gly or Ser.

- 25 Preferably, the substituting amino acid for position 196 is Asn, Asp, Gln, Glu, Pro or Ser.

Preferably, the substituting amino acid for position 197 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

- 30 Preferably, the substituting amino acid for position 198 is Asp, Gln, Glu or Ser.

Preferably, the substituting amino acid for position 199 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

Preferably, the substituting amino acid for position 200 Asn, Asp, Glu or Ser.

- 35 Preferably, the substituting amino acid for position 201 is Asp or Glu.

Preferably, the substituting amino acid for position 202 is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

Preferably, the substituting amino acid for position 203 is Ala, Asn, Asp,



Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

Preferably, the substituting amino acid for position 204 is Asn, Asp, Gln, Glu, Gly or Ser.

Preferably, the substituting amino acid for position 205 is Asn, Asp, Gln, Glu, Pro or Ser.

Preferably, the substituting amino acid for position 206 is Asp or Glu.

Preferably, the substituting amino acid for position 207 is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

Preferably, the substituting amino acid for position 208 is Ala, Asn, Asp,

Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Thr or Val.

Preferably, the substituting amino acid for position 209 is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

Preferably, the substituting amino acid for position 210 is Asp or Glu.

Preferably, the substituting amino acid for position 211 is Ala, Asn, Asp,

Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

Preferably, the substituting amino acid for position 212 is Asp, Gln, Glu or Ser.

Preferably, the substituting amino acid for position 213 is Asn, Asp, Gln, Glu, Pro or Ser.

Preferably, the substituting amino acid for position 214 is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

More preferably, the substituting amino acid for any of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 is, with reference to Table 1, is neutral or negatively charged and equally or more hydrophilic, preferably more hydrophilic, than the amino acid at the subject position in wild-type subtilisin 309.

More preferably still, the substituting amino acid for any of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 208, 209, 210, 211, 212, 213 or 214 is Asp or Glu.

#### B. Multi-Loop Regions Substitution Variants

In another embodiment of the present invention, the subtilisin 309 variant has a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, wherein the modified amino acid sequence comprises a substitution at one or more positions in one or more of the first, second, third, fourth, or fifth loop regions; whereby the subtilisin 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type subtilisin 309.

In another embodiment of the present invention, the subtilisin 309 variant further comprises one or more substitutions to the sixth loop region.

In a preferred embodiment of the present invention, the substituting amino acid for one or more of the positions in one or more of the loop regions is, with reference to Table 1, neutral or negatively charged and equally or more hydrophilic, preferably more hydrophilic, than the amino acid at the subject position in the wild-type amino acid sequence.

1. Substitutions in the First Loop Region

When a substitution occurs in the first loop region, the substitution occurs at one or more of positions 57, 58, 59, 60, 61, 63, or 64.

When a substitution occurs at position 57, the substituting amino acid is Asn, Asp, Glu or Ser.

When a substitution occurs at position 58, the substituting amino acid is Glu.

When a substitution occurs at position 59, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 60, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 61, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 63, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 64, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

2. Substitutions in the Second Loop Region

When a substitution occurs in the second loop region, the substitution occurs at one or more of positions 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, or 105.

When a substitution occurs at position 93, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

When a substitution occurs at position 94, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 95, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 96, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 97, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 98, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 99, the substituting amino acid is Asp or Glu.

5 When a substitution occurs at position 100, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 101, the substituting amino acid is Asp or Glu.

10 When a substitution occurs at position 102, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

When a substitution occurs at position 103, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 104, the substituting amino acid is Asp or Glu.

15 When a substitution occurs at position 105, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val.

### 3. Substitutions in the Third Loop Region

When a substitution occurs in the third loop region, the substitution occurs at one or more of positions 124, 125, 126, 127, 128, 129, 130 or 131.

20 When a substitution occurs at position 124, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 125, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

25 When a substitution occurs at position 126, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 127, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser.

When a substitution occurs at position 128, the substituting amino acid is Asp or Glu.

30 When a substitution occurs at position 129, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser.

When a substitution occurs at position 130, the substituting amino acid is Asp or Glu.

35 When a substitution occurs at position 131, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser, Thr.

### 4. Substitutions in the Fourth Loop Region

When a substitution occurs in the fourth loop region, the substitution occurs at one or more of positions 152, 153, 154, 155, 156, 157, 158, 159, 160

or 161.

When a substitution occurs at position 152, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 153, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 154, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 155, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 156, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 157, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 158, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 159, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 160, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 161, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

#### 5. Substitutions in the Fifth Loop Region

When a substitution occurs in the fifth loop region, the substitution occurs at one or more of positions 181, 182, 183, 184 or 185.

When a substitution occurs at position 181, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 182, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 183, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr, Tyr or Val.

When a substitution occurs at position 184, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 185, the substituting amino acid is Asn, Asp, Glu or Ser.

#### 6. Substitutions in the Sixth Loop Region

When a substitution occurs in the sixth loop region, in conjunction with one or more substitutions in one or more of the preceding five loop regions,

the substitution occurs at one or more of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214.

When a substitution occurs at position 193, the substituting amino acid  
5 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

When a substitution occurs at position 194, the substituting amino acid  
is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 195, the substituting amino acid  
is Asn, Asp, Gln, Glu, Gly or Ser.

10 When a substitution occurs at position 196, the substituting amino acid  
is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 197, the substituting amino acid  
is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

When a substitution occurs at position 198, the substituting amino acid  
15 is Asp, Gln, Glu or Ser.

When a substitution occurs at position 199, the substituting amino acid  
is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

When a substitution occurs at position 200, the substituting amino acid  
is Asn, Asp, Glu or Ser.

20 When a substitution occurs at position 201, the substituting amino acid  
is Asp or Glu.

When a substitution occurs at position 202, the substituting amino acid  
is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 203, the substituting amino acid  
25 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 204, the substituting amino acid  
is Asn, Asp, Gln, Glu, Gly or Ser.

When a substitution occurs at position 205, the substituting amino acid  
is Asn, Asp, Gln, Glu, Pro or Ser.

30 When a substitution occurs at position 206, the substituting amino acid  
is Asp or Glu.

When a substitution occurs at position 207, the substituting amino acid  
is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 208, the substituting amino acid  
35 is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 209, the substituting amino acid  
is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 210, the substituting amino acid

is Asp or Glu.

When a substitution occurs at position 211, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 212, the substituting amino acid  
5 is Asp, Gln, Glu or Ser.

When a substitution occurs at position 213, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 214, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

10 C. Additional substitutions at positions other than the loop regions

As indicated hereinbefore, in addition to the one or more substitutions made to the first, second, third, fourth, fifth and/or sixth loop regions of wild-type subtilisin 309, substitutions may be made at positions other than positions in such loop regions (hereafter referred to as "additional substitution"). In  
15 another embodiment of the present invention, the subtilisin 309 variant has a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence wherein, in addition to being substituted at one or more positions in one or more of the above-discussed loop regions, there is substitution at position 74. The additional substitution may occur at position 74 alone (preferred), or in  
20 combination with one or more additional substitutions.

Where the additional substitution to the subtilisin 309 variant is at position 74 alone, the substitution is preferably with Asn, Asp, Glu, Gly, His, Lys, Phe or Pro. Particularly preferred is where the substitution is with Asp or His.

25 Where there is more than one additional substitution to the subtilisin 309 variant, preferred is where the additional substitutions occur at position 74 in combination with one or more of the following residues: 97, 99, 101, 102, 105 and 121. Preferred additional substitution combinations include the following: 74/97; 74/99; 74/101; 74/102; 74/105; 74/121; 74/97/99; 74/97/101;  
30 74/97/102; 74/99/101; 74/99/102; 74/101/102; 74/102/105; 74/102/121; 74/105/121; 74/97/99/101; 74/97/99/102; 74/97/101/102; 74/99/101/102; 74/101/102/121; 74/102/105/121; 74/97/99/101/ 102; 74/97/101/102/121 and/or 74/97/99/101/102/121. Most preferred additional substitution combinations include the following: 74/97; 74/102; 74/97/102; 74/101/102;  
35 74/102/105; 74/99/101/102; 74/97/99/101/102 and 74/99/102.

Preferably, the additional substitutions to be made at each of the identified amino acid residue positions include but are not limited to substitutions at position 74 including Asp, His, Glu, Gly, Phe, Lys, Pro and

Asn; substitutions at position 97 including Asp, Thr, Asn, Gln, Gly and Ser; substitutions at position 99 including Gly, Asp, Lys, Leu, Ala, Glu and Ser; substitutions at position 101 including Gln, Thr, Asp, Glu, Tyr, Lys, Gly, Arg and Ser; substitutions at position 102 including Ser, Tyr, Ile, Leu, M, Ala, W, Asp, Thr, Gly and Val; substitutions at position 105 including Val, Leu, M, Tyr, Gly, Glu, Phe, Thr, Ser, Ala and Ile; and substitutions at position 121 including Asn, Thr, Ile and Ser. The specifically preferred amino acid(s) to be substituted at each such position are designated below in Table 2. Although specific amino acids are shown in Table 2, it should be understood that any amino acid may be substituted at the identified residues. As indicated herein before, these substitutions are in addition to the one or more substitutions at one or more of the loop regions, discussed above.

TABLE 2

	Amino Acid Residue	Preferred Amino Acid to be Substituted/Inserted
15	74	Asp, His
	97	Asp, Thr, Asn, Gly
	99	Arg, Gly, Asp, Lys, Leu, Ala, Glu
	101	Ala, Gln, Thr, Asp, Glu, Tyr, Lys, Gly, Arg
	102	Ile, Tyr, Ser, Leu, Ala, Thr, Gly
20	105	Val, Leu, Tyr, Gly, Phe, Thr, Ser, Ala
	121	Ser, Thr, Ile

#### D. Preparation of enzyme variants

##### Example 1

##### Mutant 309 DNA Sequences

A phagemid (pJMA602) containing the wild type subtilisin 309 (i.e., savinase) gene is constructed. The 2.8 Kbp *Pvu* II restriction enzyme fragment of plasmid pUC119, (Vieira, J. and Messing, J., "Production of Single-Stranded Plasmid DNA", 153 METHODS IN ENZYMOLOGY 3-11 (1989)) is cloned into the *Pvu* II site of plasmid pUB110 (Bacillus Genetic Stock Center, Columbus, OH 1E9). The pUC119-pUB110 hybrid plasmid is named pJMA601. Into the *Bam*H I restriction site of pJMA601 is cloned the polymerase chain reaction-amplified 309 (savinase) gene from *Bacillus lentus* chromosomal DNA (National Collections of Industrial and Marine Bacteria *Bacillus lentus* 10309) giving phagemid pJMA602. Phagemid pJMA602 is transformed into *Escherichia coli* ung-strain CJ236 and a single stranded uracil-containing DNA template is produced using the VCSM13 helper phage (Kunkel, T.A., J.D. Roberts and R.A. Zakour, "Rapid and efficient site-specific mutagenesis

without phenotypic selection", METHODS IN ENZYMOLOGY, Vol. 154, pp. 367-382, (1987); as modified by Yuckenberg, P.D., F. Witney, J. Geisselsoder and J. McClary, "Site-directed in vitro mutagenesis using uracil-containing DNA and phagemid vectors", DIRECTED MUTAGENESIS - A PRACTICAL APPROACH, ed. M.J. McPherson, pp. 27-48, (1991); both of which are incorporated herein by reference). A single primer site-directed mutagenesis modification of the method of Zoller and Smith (Zoller, M.J., and M. Smith, "Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any fragment of DNA", NUCLEIC ACIDS RESEARCH, Vol. 10, pp. 6487-6500, (1982), incorporated herein by reference) is used to produce all mutants (basically as presented by Yuckenberg, et al., 1991, above). Oligonucleotides are made using an Applied Biosystem Inc. 380B DNA synthesizer. Mutagenesis reaction products are transformed into Escherichia coli strain MM294 (American Type Culture Collection E. Coli. 33625). All mutants are confirmed by DNA sequencing and the isolated DNA is transformed into the Bacillus subtilis expression strain BG2036 (Yang, M. Y., E. Ferrari and D. J. Henner, (1984), "Cloning of the Neutral Protease Gene of Bacillus subtilis and the Use of the Cloned Gene to Create an In Vitro-derived Deletion Mutation", JOURNAL OF BACTERIOLOGY, Vol. 160, pp. 15-21). For some of the mutants a modified pJMA602 with a frameshift-stop codon mutation in the corresponding loop is used to produce the uracil template. Oligonucleotides are designed to restore the proper reading frame at position 203 and also encoded for random substitutions at positions 57, 58, 59, 60, 61, 63, 64, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 124, 125, 126, 127, 128, 129, 130, 131, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 181, 182, 183, 184, 185, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 and 214 (equimolar and/or variable mixtures of all four nucleotides for all three bases at these codons). Mutations that correct for the frameshift-stop and produce a functional enzyme are identified by their ability to digest casein. The random substitutions are determined by DNA sequencing.

#### Example 2

##### Fermentation

The Bacillus subtilis cells (BG2036) containing a subtilisin mutant of interest are grown to mid-log phase in a one liter culture of LB-glucose broth and inoculated into a Biostat ED fermenter (B. Braun Biotech, Inc., Allentown, Pennsylvania) in a total volume of 10 liters. The fermentation media contains Yeast Extract, starch, antifoam, buffers and trace minerals (see FERMENTATION:



A PRACTICAL APPROACH, Ed. B. McNeil and L. M. Harvey, 1990). The broth is kept at a constant pH of 7.0 during the fermentation run. Chloramphenicol is added for antibiotic selection of mutagenized plasmid. The cells are grown overnight at 37°C to an A<sub>600</sub> of about 60 and harvested.

### Example 3

#### Purification

The fermentation broth is taken through the following steps to obtain pure enzyme. The broth is cleared of *Bacillus subtilis* cells by centrifugation, and clarified by removing fine particulates with a 100K cutoff membrane. This is followed by concentration on a 10K cutoff membrane, and flow dialysis to reduce the ionic strength and adjust the pH to 5.5 using 0.025M MES buffer (2-(N-morpholino)ethanesulfonic acid). The enzyme is further purified by loading it onto either a cation exchange chromatography column or an affinity adsorption chromatography column and eluting it from the column with a NaCl or a propylene glycol gradient (see Scopes, R. K., PROTEIN PURIFICATION PRINCIPLES AND PRACTICE, Springer-Verlag, New York (1984), incorporated herein by reference).

The pNA assay (DeMar, E.G., C. Largman, J.W. Brodrick and M.C. Geokas, ANAL. BIOCHEM., Vol. 99, pp. 316-320, (1979), incorporated herein by reference) is used to determine the active enzyme concentration for fractions collected during gradient elution. This assay measures the rate at which p-nitroaniline is released as the enzyme hydrolyzes the soluble synthetic substrate, succinyl-alanine-alanine-proline-phenylalanine-p-nitroanilide (sAAPF-pNA). The rate of production of yellow color from the hydrolysis reaction is measured at 410 nm on a spectrophotometer and is proportional to the active enzyme concentration. In addition, absorbance measurements at 280 nm are used to determine the total protein concentration. The active enzyme/total-protein ratio gives the enzyme purity, and is used to identify fractions to be pooled for the stock solution.

To avoid autolysis of the enzyme during storage, an equal weight of propylene glycol is added to the pooled fractions obtained from the chromatography column. Upon completion of the purification procedure the purity of the stock enzyme solution is checked with SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and the absolute enzyme concentration is determined via an active site titration method using trypsin inhibitor type II-T: turkey egg white purchased from Sigma Chemical Company (St. Louis, Missouri). The measured conversion factors will show which changes made in the enzyme molecule at the various positions result in the

enzyme variant having increased activity over the wild-type, against the soluble substrate pNA.

In preparation for use, the enzyme stock solution is eluted through a Sephadex-G25 (Pharmacia, Piscataway, New Jersey) size exclusion column to remove the propylene glycol and exchange the buffer. The MES buffer in the enzyme stock solution is exchanged for 0.1 M Tris buffer (Tris(hydroxymethyl-aminomethane) containing 0.01M  $\text{CaCl}_2$  and pH adjusted to 8.6 with HCl. All experiments are carried out at pH 8.6 in Tris buffer thermostated at 25°C.

#### E. Characterization of Enzyme Variants

##### Example 4

##### Model Surface Preparation

Aminopropyl controlled pore glass (CPG) purchased from CPG Inc. (Fairfield, New Jersey) is used as a support for covalently attaching the sAAPF-pNA substrate purchased from Bachem, Inc. (Torrence, California). The reaction is carried out in dimethyl sulfoxide and (1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride) (EDC) is used as a coupling agent. Upon completion (monitored by pNA assay), the excess solvent is removed, and the CPG:sAAPF-pNA is rinsed with dimethyl sulfoxide (DMSO) and doubly-distilled water. This is followed by oven drying with a  $\text{N}_2$  purge at about 70°C. The reaction scheme and preparation of the immobilized substrate are conducted as described by Brode, P.F. III, and D.S. Rauch, "Subtilisin BPN': Activity on an Immobilized Substrate," *LANGMUIR*, Vol. 8, p. 1325-1329, (1992), incorporated herein by reference.

The CPG surface will have  $62,000 \pm 7,000$  pNA molecules/ $\mu\text{m}^2$ . The surface area will remain unchanged from the value of 50.0m<sup>2</sup>/g reported by CPG Inc. for the CPG as received. This suggests that the procedure used to add sAAPF-pNA to CPG does not damage the porous structure (mean diameter is 486 Å).

##### Example 5

##### Surface Hydrolysis Assay

Using CPG:sAAPF-pNA, adsorption of an enzyme variant and hydrolysis of a CPG-bound peptide can be measured in a single experiment. A small volume of enzyme variant stock solution is added to a flask containing Tris buffer and CPG:sAAPF-pNA which has been degassed. The flask is shaken on a wrist-action shaker for a period of 90 minutes during which the shaker is stopped at various time intervals (for example, every 2 minutes during the early stages of adsorption hydrolysis - e.g., the first 20 minutes - and every 10 minutes towards the end of the experiment). The CPG:sAAPF-

pNA is allowed to settle and the solution is sampled. Both the experimental procedure and the calculation of the adsorption and hydrolysis are conducted as described by Brode et al., 1992, above.

All enzymes are monitored for stability against autolysis and should show no appreciable autolytic loss over the time course of this experiment. Therefore, enzyme adsorption can be determined by measuring solution depletion. The difference between the initial enzyme variant concentration and the concentration measured at each individual time point gives the amount of enzyme variant adsorbed. The amount of pNA hydrolyzed from the surface is measured by taking an absorbance reading on an aliquot of the sample at 410 nm. The total amount of pNA hydrolyzed is calculated by adding the amount sampled and the amount remaining in the flask. This value is corrected by subtracting the amount of pNA that is hydrolyzed by Tris buffer at pH 8.6 when no enzyme is present. This base-hydrolysis ranges from 7-29% of the total hydrolysis depending on the efficiency of the enzyme.

#### Example 6

##### Soluble Substrate Kinetic Analysis

The rates of hydrolysis of the soluble substrate sAAPF-pNA are monitored by measuring the adsorbance increase as a function of time at 410 nm on a DU-70 spectrophotometer. The enzyme concentration is held constant and is prepared to be in the range of 6-10 nanomolar while the substrate concentration is varied from 90-700  $\mu$ M sAAPF-pNA for each kinetic determination. An adsorbance data point is taken each second over a period of 900 seconds and the data are transferred to a LOTUS™ spreadsheet (Lotus Development Corporation, Cambridge, Massachusetts). Analysis for kinetic parameters is conducted by the standard Lineweaver Burk analysis in which the data in the initial part of the run (generally the first minute) are fit to a linear regression curve to give  $v_0$ . The  $v_0$  and  $s_0$  data are plotted in the standard inverse fashion to give  $K_M$  and  $k_{cat}$ .

#### 30 F. Example Subtilisin 309 Variants

Subtilisin 309 variants of the present invention which have decreased adsorption to and increased hydrolysis of surface bound substrates are exemplified in Tables 3-38, below. In describing the specific mutations, the original amino acid occurring in wild-type is given first, the position number second, and the substituted amino acid third.

TABLE 3

Loop 1 - Single Mutation Variants
Gln57Asn

22

	Gln57Asp
	Gln57Glu
	Gln57Ser
	Asp58Glu
5	Gly59Asn
	Gly59Asp
	Gly59Gln
	Gly59Glu
	Gly59Pro
10	Gly59Ser
	Asn60Asp
	Asn60Gln
	Asn60Glu
	Asn60Ser
15	Gly61Asn
	Gly61Asp
	Gly61Gln
	Gly61Glu
	Gly61Pro
20	Gly61Ser
	Gly63Asn
	Gly63Asp
	Gly63Gln
	Gly63Glu
25	Gly63Pro
	Gly63Ser
	Thr64Asn
	Thr64Asp
	Thr64Gln
30	Thr64Glu
	Thr64Gly
	Thr64Pro
	Thr64Ser

TABLE 4

## Loop 1 - Double Mutation Variants

	Gln57Ser + Asn60Glu
	Asp58Glu + Gly61Gln
	Gly59Ser + Gly63Ser
40	Asn60Ser + Gly61Ser
	Gly63Asn + Thr64Asp
	Gly59Asn + Thr64Glu
	Asn60Glu + Gly63Ser
	Asn60Gln + Gly63Gln
45	Asn60Asp + Gly63Ser
	Asp58Glu + Gly63Gln
	Gln57Asp + Thr64Gly
	Gln57Glu + Gly63Gln
	Asn60Glu + Gly63Asn
50	Gly61Gln + Gly63Asn
	Asp58Glu + Gly59Asn
	Asp58Glu + Thr64Pro

	Gly61Asn + Gly63Asn
	Asp58Glu + Gly61Pro
	Asn60Glu + Gly63Gln
	Asn60Asp + Gly61Gln
5	Asp58Glu + Asn60Ser
	Gln57Asn + Gly59Glu
	Asn60Glu + Gly61Pro
	Gly61Asn + Thr64Gln
	Asp58Glu + Thr64Ser
10	Asn60Asp + Thr64Gln
	Gly59Glu + Gly63Ser
	Gln57Asn + Gly61Ser
	Gly61Ser + Thr64Asn
	Gln57Asp + Gly61Asn
15	Gly59Glu + Gly63Pro
	Asn60Gln + Gly61Ser
	Gly61Pro + Gly63Ser
	Gly59Asp + Gly63Asn
	Gly63Gln + Thr64Asn
20	Gln57Glu + Gly59Gln
	Gln57Asp + Gly59Pro
	Gln57Glu + Gly61Ser
	Gln57Asp + Gly63Gln
	Asp58Glu + Gly61Asn
25	Gly59Asn + Asn60Glu
	Gly61Gln + Thr64Glu
	Gln57Glu + Gly61Gln
	Asp58Glu + Gly63Ser
	Gly59Glu + Gly63Asn
30	Gly61Ser + Thr64Asp
	Gly59Ser + Thr64Asp
	Gln57Glu + Gly63Pro
	Gly59Ser + Thr64Glu
	Asn60Gln + Gly63Pro
35	Gln57Asn + Asn60Asp

TABLE 5

Loop 1 - Triple Mutation Variants	
40	Gly59Pro + Gly63Gln + Thr64Glu
	Gln57Ser + Gly59Asn + Gly63Pro
	Gly59Pro + Asn60Asp + Thr64Asn
	Gln57Glu + Gly59Asn + Gly63Pro
	Gln57Asp + Gly63Pro + Thr64Asn
	Gln57Ser + Asn60Glu + Thr64Gly
45	Gln57Ser + Asn60Ser + Gly63Ser
	Gly61Ser + Gly63Ser + Thr64Gln
	Gln57Glu + Gly61Pro + Thr64Gly
	Asp58Glu + Gly63Pro + Thr64Gly
	Gln57Asn + Asp58Glu + Gly61Pro
50	Asp58Glu + Gly59Ser + Thr64Pro
	Gln57Asn + Gly59Glu + Gly61Ser
	Gly59Glu + Gly61Gln + Thr64Asn

5 Gln57Asp + Asn60Gln + Thr64Gly  
 Asn60Glu + Gly61Gln + Thr64Gly  
 Gln57Asp + Gly59Pro + Gly63Pro  
 Gln57Asn + Gly63Asn + Thr64Gly  
 Gln57Ser + Gly59Asp + Gly61Ser  
 Gly61Gln + Gly63Asn + Thr64Pro  
 Gln57Glu + Gly59Pro + Thr64Ser  
 Asn60Asp + Gly63Gln + Thr64Gly  
 10 Asp58Glu + Gly63Asn + Thr64Gln  
 Asp58Glu + Gly61Gln + Gly63Gln  
 Gln57Asp + Gly61Pro + Thr64Gln  
 Asp58Glu + Gly59Asn + Gly61Ser  
 Asp58Glu + Gly59Ser + Asn60Ser  
 Gln57Asn + Gly61Pro + Thr64Gly  
 15 Gly59Glu + Gly61Pro + Gly63Pro  
 Gly59Glu + Gly61Gln + Gly63Asn  
 Gly59Glu + Gly61Pro + Gly63Gln  
 Gly59Glu + Gly61Pro + Thr64Asn  
 Gln57Asn + Asp58Glu + Thr64Gln

20

TABLE 6

## Loop 1 - Quadruple Mutation Variants

25 Gly59Gln + Asn60Asp + Gly61Pro + Gly63Pro  
 Gly59Ser + Asn60Gln + Gly63Ser + Thr64Asp  
 Gly59Pro + Asn60Ser + Gly61Ser + Gly63Gln  
 Gln57Glu + Gly59Pro + Asn60Ser + Gly61Pro  
 Gln57Glu + Asn60Gln + Gly63Asn + Thr64Gly  
 Gln57Ser + Gly59Ser + Asn60Asp + Gly61Ser  
 Gln57Ser + Asn60Glu + Gly61Pro + Thr64Pro  
 30 Asn60Gln + Gly61Pro + Gly63Gln + Thr64Asp  
 Asn60Glu + Gly61Gln + Gly63Asn + Thr64Pro  
 Gln57Glu + Gly59Gln + Gly61Pro + Thr64Gln  
 Gln57Asn + Asp58Glu + Gly59Ser + Asn60Gln  
 Asp58Glu + Asn60Ser + Gly63Ser + Thr64Ser  
 35 Gln57Glu + Gly59Pro + Gly63Gln + Thr64Ser  
 Asp58Glu + Gly59Ser + Asn60Ser + Gly63Ser  
 Gly59Asn + Asn60Asp + Gly61Ser + Thr64Gln  
 Gln57Glu + Asp58Glu + Gly59Gln + Gly61Gln  
 Gln57Glu + Asp58Glu + Asn60Ser + Thr64Pro  
 40 Gln57Asp + Asp58Glu + Gly61Ser + Gly63Pro  
 Gln57Asp + Asp58Glu + Asn60Gln + Gly61Gln  
 Gln57Ser + Asp58Glu + Gly59Glu + Asn60Gln  
 Asp58Glu + Gly59Asp + Gly61Ser + Gly63Gln  
 Gly59Glu + Asn60Asp + Gly61Asn + Gly63Asn  
 45 Gly59Asp + Asn60Asp + Gly61Asn + Gly63Gln  
 Gly59Glu + Asn60Asp + Gly61Pro + Thr64Ser  
 Asp58Glu + Gly59Glu + Asn60Asp + Thr64Gly  
 Asp58Glu + Gly59Glu + Asn60Asp + Gly61Pro  
 Asp58Glu + Gly59Glu + Asn60Glu + Gly61Asn

50

TABLE 7

Loop 2 - Single Mutation Variants	
5	Val 93Ala
	Val 93Asn
	Val 93Asp
	Val 93Cys
	Val 93Gln
	Val 93Glu
10	Val 93Gly
	Val 93His
	Val 93Met
	Val 93Pro
	Val 93Ser
	Val 93Thr
15	Leu 94Ala
	Leu 94Asn
	Leu 94Asp
	Leu 94Cys
	Leu 94Gln
	Leu 94Glu
20	Leu 94Gly
	Leu 94His
	Leu 94Ile
	Leu 94Met
	Leu 94Pro
	Leu 94Ser
25	Leu 94Thr
	Leu 94Val
	Gly 95Asn
	Gly 95Asp
	Gly 95Gln
	Gly 95Glu
30	Gly 95Pro
	Gly 95Ser
	Ala 96Asn
	Ala 96Asp
	Ala 96Gln
	Ala 96Glu
35	Ala 96Gly
	Ala 96His
	Ala 96Pro
	Ala 96Ser
	Ala 96Thr
	Ser 97Asp
40	Ser 97Glu
	Gly 98Asn
	Gly 98Asp
	Gly 98Gln
	Gly 98Glu
	Gly 98Pro
45	Gly 98Ser
	Ser 99Asp
	Ser 99Glu
50	

	Gly100Asn
	Gly100Asp
	Gly100Gln
	Gly100Glu
5	Gly100Pro
	Gly100Ser
	Ser101Asp
	Ser101Glu
	Val102Ala
10	Val102Asn
	Val102Asp
	Val102Cys
	Val102Gln
	Val102Glu
15	Val102Gly
	Val102His
	Val102Met
	Val102Pro
	Val102Ser
20	Val102Thr
	Ser103Asp
	Ser103Glu
	Ser104Asp
	Ser104Glu
25	Ile105Ala
	Ile105Asn
	Ile105Asp
	Ile105Cys
	Ile105Gln
30	Ile105Glu
	Ile105Gly
	Ile105His
	Ile105Leu
	Ile105Met
35	Ile105Pro
	Ile105Ser
	Ile105Thr
	Ile105Val

TABLE 8

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 Loop 2 - Double Mutation Variants
 

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	Val 93Gln + Ser 99Glu
	Gly 95Ser + Gly 98Gln
	Ser101Asp + Ile105Ala
45	Leu 94Ser + Gly 95Ser
	Leu 94Pro + Ser101Asp
	Gly 98Gln + Ser103Asp
	Ser 97Asp + Tyr102Gln
	Tyr102Cys + Ile105Met
50	Val 93Pro + Gly 98Gln
	Ser 99Glu + Gly100Pro
	Ser103Asp + Ile105Leu



5 Ser 97Asp + Gly 98Gln  
Val 93Ser + Ser 99Asp  
Leu 94Ser + Gly 98Glu  
Gly 95Glu + Tyr102Met  
Gly 95Asn + Ser103Glu  
Gly 98Ser + Tyr102Thr  
Gly 98Gln + Tyr102Cys  
Leu 94Ile + Ile105Gln  
Leu 94Asp + Gly100Gln  
10 Ala 96Gly + Ser101Asp  
Ser101Glu + Ile105Gln  
Gly 95Gln + Ile105Ser  
Val 93Gln + Gly100Pro  
Val 93Met + Ser104Asp  
15 Gly 98Pro + Ser101Asp  
Val 93Pro + Ile105His  
Gly 95Asp + Gly100Ser  
Tyr102Cys + Ile105Leu  
Gly100Asn + Ile105Met  
20 Gly 98Asn + Ser101Glu  
Ser 99Glu + Ile105Gln  
Val 93Thr + Tyr102Thr  
Gly 98Ser + Ser104Glu  
Gly 95Asn + Ser104Glu  
25 Val 93Pro + Ser103Glu  
Gly 95Asn + Tyr102Ile  
Leu 94Pro + Gly 98Asp  
Leu 94His + Gly 98Asp  
Val 93Asn + Tyr102Thr  
30 Tyr102Ala + Ser103Asp  
Gly 98Pro + Ser103Glu  
Leu 94Cys + Tyr102Leu  
Val 93Gly + Gly 98Ser  
Gly100Gln + Tyr102Ser  
35 Val 93Thr + Gly100Asn  
Gly 98Asn + Ile105Pro  
Val 93Asp + Leu 94Ala  
Leu 94Gly + Ser104Glu  
Val 93Met + Ser101Asp  
40 Val 93Met + Ser 97Glu  
Ala 96Pro + Ile105Asn  
Ser 97Glu + Gly 98Pro  
Ala 96Thr + Ser 99Glu  
Val 93Asn + Gly100Asn  
45 Gly 95Gln + Gly 98Pro  
Gly 95Asn + Ala 96His  
Val 93Ser + Ser103Asp  
Gly 98Gln + Ile105Pro  
Val 93Cys + Ile105Glu

---

TABLE 9

Loop 2 - Triple Mutation Variants	
5	Val 93Gln + Leu 94Thr + Ser 99Glu
	Leu 94Met + Gly 95Gln + Ser104Asp
	Ser 99Glu + Tyr102Met + Ile105Thr
	Val 93Thr + Leu 94Thr + Ile105Cys
	Leu 94Asp + Ala 96Thr + Gly 98Asn
10	Val 93Met + Gly 95Gln + Ser103Asp
	Val 93Cys + Ala 96Pro + Ser 99Glu
	Leu 94Asp + Ala 96Gly + Gly100Gln
	Leu 94His + Gly 98Gln + Ser 99Glu
	Gly 95Pro + Ser101Asp + Tyr102Gln
15	Leu 94Asn + Gly 95Gln + Ser101Asp
	Leu 94His + Gly 98Pro + Ser104Glu
	Leu 94Thr + Ala 96Asn + Gly100Pro
	Gly 95Ser + Gly 98Ser + Tyr102Glu
	Ala 96Thr + Ser 97Asp + Tyr102Thr
20	Leu 94Asn + Gly100Ser + Ser101Glu
	Leu 94Met + Gly 98Gln + Gly100Asn
	Val 93Pro + Ala 96Glu + Ile105Ala
	Val 93Cys + Gly 95Glu + Tyr102Leu
	Leu 94Cys + Gly 95Ser + Ser 97Asp
25	Gly 95Gln + Ser101Glu + Tyr102His
	Val 93Gln + Gly 95Glu + Ile105Gln
	Val 93Gly + Ser 99Asp + Ile105Gly
	Gly 95Asn + Gly 98Glu + Ile105Val
	Ser 97Glu + Gly 98Pro + Tyr102Thr
30	Val 93Glu + Leu 94Ile + Gly100Gln
	Ala 96Pro + Ser101Asp + Ile105Pro
	Gly 98Pro + Ser103Asp + Ile105Val
	Ala 96Gln + Ser 99Asp + Tyr102Ser
	Ser 97Asp + Gly 98Ser + Ile105Asn
35	Val 93Gln + Gly 98Asn + Tyr102Leu
	Leu 94Gly + Tyr102Pro + Ser103Glu
	Val 93Thr + Gly100Gln + Ser104Glu
	Val 93Gly + Gly100Gln + Ser103Glu
	Ala 96Thr + Gly100Ser + Ser103Glu
40	Gly100Asn + Ser101Glu + Ile105Thr
	Gly 95Asp + Tyr102Gly + Ile105Met
	Val 93Cys + Ala 96Thr + Ile105Cys
	Ala 96Gly + Gly 98Asp + Ser 99Asp
	Gly 95Ser + Ser 97Asp + Gly 98Asp
45	Leu 94Asn + Ser103Glu + Ser104Asp
	Tyr102Val + Ser103Glu + Ser104Glu
	Ser 99Asp + Gly100Glu + Ile105Met
	Ser 97Asp + Gly 98Asp + Ser 99Glu
	Gly 95Asn + Ser101Glu + Ser103Glu
50	Ser101Glu + Tyr102Asn + Ser103Glu
	Leu 94Asp + Ser 99Glu + Gly100Gln
	Gly100Asp + Ser101Glu + Ser104Glu
	Leu 94Asp + Gly 98Glu + Ile105Cys
	Gly 95Glu + Ser 97Glu + Tyr102Asn
	Tyr102Thr + Ser103Glu + Ile105Glu

	Gly 95Asp + Ser 99Glu + Gly100Gln
	Gly 95Glu + Ser 99Glu + Tyr102Met
	Val 93Gln + Ser 97Asp + Ser 99Glu
	Gly100Glu + Ser101Asp + Ser103Asp
5	Val 93Asp + Ser103Glu + Ser104Glu
	Val 93Asp + Ser 99Asp + Gly100Asp
	Ala 96Gln + Gly100Asp + Ser104Asp
	Val 93Asp + Ser101Glu + Ile105Asp
	Leu 94Asp + Ser 99Glu + Ser101Glu

10

TABLE 10

Loop 2 - Quadruple Mutation Variants	
	Leu 94Asn + Ser 97Glu + Tyr102Asn + Ile105Met
	Leu 94Pro + Gly 95Glu + Gly100Gln + Tyr102Ala
15	Val 93Cys + Leu 94Met + Gly 95Pro + Ala 96Gln
	Gly 95Pro + Ser 97Glu + Tyr102Gln + Ile105Leu
	Gly 95Asp + Gly100Pro + Tyr102Val + Ile105Cys
	Ala 96Thr + Gly100Asp + Tyr102Ala + Ile105Asn
	Val 93Asn + Leu 94Cys + Ser101Asp + Tyr102Val
20	Val 93Asn + Gly 95Asn + Ser104Asp + Ile105Val
	Leu 94Ser + Gly 98Pro + Ser103Glu + Ile105Val
	Val 93Asn + Leu 94His + Gly 95Asn + Ile105Asn
	Ala 96Ser + Gly 98Asn + Ser 99Glu + Tyr102Gly
	Val 93Asn + Gly 98Asn + Tyr102Ile + Ile105Asp
25	Val 93Asn + Tyr102Asn + Ser103Asp + Ile105Ser
	Ala 96Ser + Gly100Ser + Ser101Asp + Tyr102Pro
	Leu 94Pro + Ala 96Ser + Ser101Asp + Tyr102Ser
	Val 93Met + Ala 96Thr + Ser104Asp + Ile105Gln
	Val 93Met + Leu 94Ala + Gly 95Pro + Ser104Glu
30	Val 93Cys + Gly 95Pro + Ala 96Glu + Tyr102Val
	Gly 95Asn + Ala 96Asn + Ser104Glu + Ile105Pro
	Val 93Asn + Leu 94Asn + Gly 98Glu + Ser 99Glu
	Leu 94His + Gly 98Glu + Ser 99Glu + Tyr102Ser
	Gly 95Pro + Gly 98Glu + Ser 99Asp + Ile105Gly
35	Val 93Ala + Ala 96Ser + Ser103Glu + Ser104Glu
	Leu 94Gly + Ser 97Asp + Gly 98Asp + Ser 99Glu
	Val 93Cys + Leu 94Asp + Gly 95Asn + Gly100Glu
	Val 93Ser + Ser101Glu + Ser103Asp + Ser104Asp
	Ala 96Ser + Ser101Glu + Tyr102Gln + Ser103Asp
40	Val 93Thr + Gly100Asn + Ser101Glu + Ser103Asp
	Val 93Asn + Leu 94Glu + Gly 95Glu + Ser 99Glu
	Leu 94Asp + Ala 96Gly + Ser 99Glu + Ile105Cys
	Leu 94Ala + Ser101Glu + Ser104Asp + Ile105Ser
	Ala 96His + Ser101Glu + Ser104Glu + Ile105Ala
45	Val 93Gly + Gly100Pro + Ser101Glu + Ser104Asp
	Gly 95Gln + Ser101Glu + Ser104Asp + Ile105Met
	Gly 98Gln + Ser101Glu + Ser104Glu + Ile105Asn
	Val 93Gly + Ser101Asp + Tyr102Glu + Ile105Asp
	Ser101Asp + Tyr102Val + Ser104Glu + Ile105Glu
50	Val 93Cys + Gly 95Asp + Gly 98Asp + Gly100Pro
	Gly 95Asp + Ser 97Glu + Tyr102Ala + Ile105Cys
	Val 93Thr + Gly 95Glu + Ala 96Asn + Ser 99Asp

	Val 93Cys + Gly 95Asp + Ser 99Glu + Ile105Val
	Val 93Cys + Ser101Asp + Ser103Glu + Ile105Asp
	Ala 96His + Ser 97Glu + Ser 99Asp + Ile105Asn
	Ala 96Asn + Ser 97Glu + Ser 99Glu + Tyr102Pro
5	Val 93Cys + Ser 97Glu + Ser 99Asp + Ile105His
	Val 93Ser + Ser 97Glu + Gly 98Gln + Ser 99Glu
	Gly 95Asn + Ser 97Asp + Ser 99Glu + Gly100Glu
	Val 93Met + Gly100Asp + Tyr102Asp + Ser103Asp
	Leu 94Gly + Ser101Asp + Tyr102Ala + Ile105Glu
10	Ser 97Glu + Gly 98Asp + Gly100Asp + Tyr102Gly
	Leu 94Ser + Ser 99Glu + Gly100Glu + Ser104Glu
	Val 93Asn + Ser 99Glu + Gly100Glu + Ser104Asp
	Val 93Ala + Ser 99Glu + Gly100Asp + Ser104Asp
	Leu 94Cys + Gly 95Ser + Ser 99Asp + Ser101Glu
15	Gly 95Ser + Ala 96Thr + Ser 99Glu + Ser101Asp
	Leu 94Cys + Ala 96Ser + Ser 99Asp + Ser101Asp
	Ser 99Asp + Ser101Glu + Tyr102Asn + Ile105Val
	Val 93Glu + Gly 95Pro + Ser101Glu + Ser103Asp
	Val 93Ala + Gly 95Asp + Ser 99Asp + Ser101Glu
20	Ser 99Glu + Ser101Glu + Tyr102Pro + Ser104Glu

TABLE 11

## Loop 3 - Single Mutation Variants

	Leu124Ala
25	Leu124Asn
	Leu124Asp
	Leu124Cys
	Leu124Gln
	Leu124Glu
30	Leu124Gly
	Leu124His
	Leu124Ile
	Leu124Met
	Leu124Pro
35	Leu124Ser
	Leu124Thr
	Leu124Val
	Gly125Asn
	Gly125Asp
40	Gly125Gln
	Gly125Glu
	Gly125Pro
	Gly125Ser
	Ser126Asp
45	Ser126Glu
	Pro127Asn
	Pro127Asp
	Pro127Gln
	Pro127Glu
50	Pro127Gly
	Pro127Ser
	Ser128Asp

5 Ser128Glu  
 Pro129Asn  
 Pro129Asp  
 Pro129Gln  
 Pro129Glu  
 Pro129Gly  
 Pro129Ser  
 Ser130Asp  
 Ser130Glu  
 10 Ala131Asn  
 Ala131Asp  
 Ala131Gln  
 Ala131Glu  
 Ala131Gly  
 15 Ala131His  
 Ala131Pro  
 Ala131Ser  
 Ala131Thr

TABLE 12

## Loop 3 - Double Mutation Variants

20  
 25 Leu124Gln + Ser128Glu  
 Gly125Ser + Ser126Glu  
 Gly125Ser + Ser128Glu  
 Pro127Gly + Ser130Glu  
 Leu124Thr + Ser126Glu  
 Ser126Glu + Pro129Ser  
 Gly125Gln + Ser128Glu  
 Pro127Ser + Ser130Glu  
 30 Gly125Glu + Pro127Asn  
 Pro129Gln + Ser130Glu  
 Gly125Asp + Ala131His  
 Pro127Gly + Ala131Glu  
 Leu124His + Ser126Asp  
 35 Pro127Gly + Pro129Asp  
 Ser126Glu + Ala131Gln  
 Leu124Asp + Ala131Pro  
 Gly125Pro + Pro127Glu  
 Gly125Ser + Ser126Asp  
 40 Leu124Gln + Ala131Asn  
 Gly125Asp + Pro129Ser  
 Pro129Asn + Ala131Asp  
 Pro127Asn + Ser128Asp  
 Pro129Gly + Ala131Glu  
 45 Pro127Ser + Pro129Asp  
 Pro127Ser + Ser128Glu  
 Leu124Gly + Ser130Glu  
 Gly125Asn + Ser130Asp  
 Leu124Glu + Pro127Asn  
 50 Ser130Glu + Ala131Gly  
 Ser130Asp + Ala131Thr  
 Leu124Gln + Ser128Asp

	Leu124Ser + Ala131Pro
	Pro129Ser + Ser130Glu
	Gly125Gln + Pro127Asp
	Leu124Cys + Ala131Thr
5	Gly125Gln + Pro129Gln
	Leu124Met + Ala131Gln
	Gly125Gln + Ala131Asp
	Ser128Glu + Ala131Asn
	Ser130Glu + Ala131Pro
10	Gly125Pro + Ala131Ser
	Pro127Ser + Pro129Asn
	Ser126Glu + Pro127Gln
	Pro127Ser + Ser130Asp
	Pro127Gly + Ser128Asp
15	Gly125Glu + Pro127Ser
	Leu124Cys + Pro129Asn
	Gly125Gln + Pro129Glu
	Gly125Asn + Ala131Asp
	Gly125Ser + Pro129Gln
20	Ser126Asp + Pro129Gln
	Leu124Val + Pro129Asn
	Leu124His + Ser126Glu
	Pro127Glu + Ala131Gly
	Leu124Thr + Gly125Pro
25	Leu124His + Ser130Glu
	Gly125Asn + Ser126Asp
	Pro129Asn + Ala131Pro
	Pro127Gln + Pro129Asn
	Leu124Gly + Pro129Glu

30

TABLE 13

Loop 3 - Triple Mutation Variants	
	Gly125Gln + Ser126Glu + Ala131His
	Ser126Asp + Pro127Ser + Ala131His
35	Leu124Val + Ser130Asp + Ala131Asn
	Leu124Gln + Ser126Glu + Ala131Ser
	Gly125Ser + Pro129Glu + Ala131Gly
	Leu124Ser + Pro127Asp + Ala131Gln
	Pro127Ser + Ser128Asp + Ala131His
40	Leu124Thr + Pro127Glu + Ala131Ser
	Leu124Gln + Ser126Glu + Ala131Asn
	Gly125Asn + Pro127Glu + Ala131Gln
	Gly125Gln + Ser126Asp + Pro127Gln
	Pro127Asn + Ser130Asp + Ala131Thr
45	Gly125Asn + Pro127Glu + Pro129Asn
	Leu124Thr + Ser128Asp + Ala131Gln
	Gly125Ser + Pro129Gln + Ala131Glu
	Pro127Gln + Pro129Asn + Ser130Glu
	Leu124Ile + Gly125Gln + Ser130Glu
50	Pro129Asn + Ser130Glu + Ala131Gly
	Leu124Ser + Gly125Ser + Pro129Gln
	Leu124Asn + Gly125Gln + Ser128Asp

	Leu124Gln + Pro127Ser + Ser130Asp
	Gly125Ser + Pro127Ser + Ala131Asp
	Gly125Ser + Ser128Glu + Pro129Gln
	Pro127Asn + Ser128Asp + Ala131Gly
5	Leu124Thr + Gly125Ser + Ala131Asn
	Gly125Gln + Pro129Ser + Ser130Asp
	Gly125Pro + Ser126Glu + Pro129Asn
	Pro129Gly + Ser130Asp + Ala131Pro
	Gly125Asn + Ser126Asp + Pro129Gly
10	Gly125Asp + Ser126Asp + Ala131Asn
	Gly125Glu + Ser126Glu + Ala131Ser
	Gly125Glu + Ser126Asp + Ala131His
	Gly125Gln + Ser128Glu + Pro129Glu
	Ser128Glu + Pro129Glu + Ala131Gly
15	Leu124Cys + Ser128Glu + Pro129Asp
	Pro127Gly + Ser128Glu + Pro129Glu
	Pro127Gly + Ser128Asp + Pro129Asp
	Leu124Asn + Ser128Glu + Pro129Asp
	Ser126Glu + Pro127Asp + Ala131Asn
20	Ser126Glu + Pro127Glu + Pro129Gly
	Leu124His + Ser126Asp + Pro127Glu
	Ser126Asp + Pro127Glu + Pro129Gly
	Pro127Asp + Ser128Asp + Pro129Asn
	Pro127Asp + Ser128Glu + Ala131His
25	Pro127Glu + Ser128Asp + Ala131Pro
	Leu124Ile + Pro127Glu + Ser128Glu
	Pro129Asp + Ser130Asp + Ala131Gly
	Leu124Pro + Pro129Glu + Ser130Glu
	Ser126Asp + Pro127Glu + Ser128Asp
30	Ser126Glu + Pro127Asp + Ser128Asp
	Pro129Glu + Ser130Asp + Ala131Glu
	Leu124Asp + Gly125Asp + Ser126Asp
	Leu124Ser + Ser126Asp + Ser128Asp
	Ser126Glu + Ser128Asp + Pro129Asn
35	Gly125Asn + Ser126Glu + Ser128Glu
	Ser126Glu + Ser128Glu + Ala131Thr
	Leu124Asn + Ser126Glu + Ser128Glu
	Ser126Glu + Ser128Glu + Pro129Glu
	Gly125Glu + Ser126Glu + Ser128Asp
40	Ser128Asp + Pro129Ser + Ser130Glu

TABLE 14

Loop 3 - Quadruple Mutation Variants	
45	Gly125Gln + Ser126Glu + Pro127Ser + Ala131Ser
	Gly125Asn + Pro127Ser + Ser130Asp + Ala131Asn
	Leu124Ala + Pro127Gln + Ser128Glu + Ala131Pro
	Gly125Pro + Pro127Ser + Pro129Ser + Ser130Glu
	Gly125Asn + Pro129Asn + Ser130Asp + Ala131Gln
	Gly125Asn + Pro129Asn + Ser130Glu + Ala131Pro
50	Leu124Ser + Ser126Glu + Pro129Gly + Ala131Gln
	Gly125Asn + Ser126Glu + Pro127Gly + Ala131Ser
	Leu124Ile + Ser128Asp + Pro129Gln + Ala131Ser

	Leu124His + Gly125Ser + Pro129Gly + Ala131Asn
	Leu124Ala + Gly125Glu + Pro127Gln + Pro129Gln
	Leu124Ser + Gly125Glu + Ser126Asp + Pro129Ser
	Gly125Glu + Ser126Glu + Pro127Gln + Ala131His
5	Leu124Met + Gly125Asp + Ser126Asp + Pro127Asn
	Leu124Gln + Ser128Glu + Pro129Asp + Ala131Gln
	Leu124Val + Ser128Glu + Pro129Glu + Ala131Asn
	Pro127Ser + Ser128Asp + Pro129Asp + Ala131Thr
	Leu124Ile + Ser128Glu + Pro129Asp + Ala131Ser
10	Gly125Ser + Ser126Glu + Pro127Glu + Ala131Gln
	Leu124His + Gly125Gln + Ser130Glu + Ala131Glu
	Leu124Thr + Gly125Gln + Ser130Asp + Ala131Asp
	Leu124His + Pro127Glu + Ser128Glu + Ala131His
	Gly125Ser + Pro127Asp + Ser128Asp + Pro129Gln
15	Leu124Asn + Ser126Glu + Pro127Asp + Ser128Asp
	Pro127Gln + Ser128Glu + Pro129Asp + Ser130Asp
	Gly125Pro + Ser128Asp + Pro129Glu + Ser130Glu
	Leu124Asn + Gly125Asp + Ser126Asp + Pro127Asp
	Leu124Asp + Gly125Asp + Ser126Asp + Pro129Gln
20	Leu124Asp + Gly125Glu + Ser126Asp + Pro129Gly
	Gly125Gln + Ser126Glu + Ser128Glu + Ala131Thr
	Leu124Asn + Ser126Glu + Pro127Gln + Ser128Asp
	Leu124Met + Gly125Pro + Ser126Asp + Ser128Glu
	Leu124Asn + Gly125Ser + Ser126Asp + Ser128Asp
25	Leu124Val + Ser126Glu + Ser128Glu + Pro129Asp
	Leu124Cys + Ser126Asp + Ser128Glu + Pro129Glu
	Ser126Asp + Pro127Asp + Pro129Glu + Ala131Ser
	Gly125Asn + Ser126Glu + Pro127Glu + Pro129Glu
	Leu124Ser + Ser126Asp + Pro127Asp + Pro129Asp
30	Gly125Asp + Ser126Glu + Ser128Asp + Pro129Ser
	Leu124Val + Gly125Asp + Ser126Glu + Ser128Glu
	Gly125Glu + Ser126Glu + Ser128Asp + Pro129Asn
	Gly125Glu + Ser126Asp + Pro127Ser + Ser128Asp
	Leu124Met + Ser128Glu + Ser130Glu + Ala131Ser
35	Leu124Cys + Ser128Glu + Pro129Ser + Ser130Asp
	Gly125Gln + Ser128Glu + Ser130Glu + Ala131His
	Leu124Met + Pro127Gln + Ser128Glu + Ser130Glu
	Leu124Gly + Ser126Asp + Pro129Glu + Ser130Asp
	Gly125Asn + Ser126Glu + Pro129Glu + Ser130Glu
40	Leu124Gly + Gly125Asp + Pro127Asp + Pro129Gly
	Ser126Asp + Ser128Asp + Ser130Glu + Ala131Gln
	Gly125Pro + Ser126Asp + Ser128Glu + Ser130Asp
	Ser126Asp + Pro127Gly + Ser128Glu + Ser130Glu
	Ser126Glu + Pro127Gly + Ser128Asp + Ser130Glu
45	Ser126Asp + Ser128Asp + Pro129Ser + Ser130Asp
	Ser126Glu + Ser128Asp + Ser130Asp + Ala131His
	Ser126Glu + Ser128Glu + Ser130Glu + Ala131Ser
	Gly125Pro + Ser126Glu + Ser128Glu + Ser130Asp
	Leu124Val + Ser126Asp + Ser128Glu + Ser130Asp
50	Gly125Gln + Ser126Glu + Ser128Asp + Ser130Glu
	Ser126Glu + Ser128Asp + Pro129Gly + Ser130Glu



TABLE 15

Loop 4 - Single Mutation Variants	
5	Gly152Asn
	Gly152Asp
	Gly152Gln
	Gly152Glu
	Gly152Pro
	Gly152Ser
10	Asn153Asp
	Asn153Gln
	Asn153Glu
	Asn153Ser
15	Ser154Asp
	Ser154Glu
	Gly155Asn
	Gly155Asp
	Gly155Gln
	Gly155Glu
20	Gly155Pro
	Gly155Ser
	Ala156Asn
	Ala156Asp
	Ala156Gln
25	Ala156Glu
	Ala156Gly
	Ala156His
	Ala156Pro
	Ala156Ser
	Ala156Thr
30	Gly157Asn
	Gly157Asp
	Gly157Gln
	Gly157Glu
	Gly157Pro
35	Gly157Ser
	Ser158Asp
	Ser158Glu
	Ile159Ala
40	Ile159Asn
	Ile159Asp
	Ile159Cys
	Ile159Gln
	Ile159Glu
	Ile159Gly
45	Ile159His
	Ile159Leu
	Ile159Met
	Ile159Pro
	Ile159Ser
50	Ile159Thr
	Ile159Val
	Ser160Asp
	Ser160Glu

36

	Tyr161Ala
	Tyr161Asn
	Tyr161Asp
	Tyr161Cys
5	Tyr161Gln
	Tyr161Glu
	Tyr161Gly
	Tyr161His
	Tyr161Ile
10	Tyr161Leu
	Tyr161Met
	Tyr161Pro
	Tyr161Ser
	Tyr161Thr
15	Tyr161Val

TABLE 16

## Loop 4 - Double Mutation Variants

	Ser154Glu + Gly155Asn
20	Ser154Asp + Gly157Gln
	Ser154Glu + Ile159His
	Gly152Gln + Gly157Asp
	Ser154Glu + Gly155Ser
	Asn153Ser + Ser154Glu
25	Ala156Asn + Ser160Glu
	Ala156Thr + Ser158Glu
	Asn153Glu + Gly157Ser
	Gly155Asn + Ala156Asp
	Ile159Gly + Tyr161Gly
30	Gly155Ser + Tyr161Glu
	Ser154Asp + Gly155Pro
	Gly155Asp + Ala156Ser
	Gly152Glu + Asn153Ser
	Ser158Glu + Ile159Asn
35	Asn153Asp + Ala156His
	Ser154Glu + Tyr161Cys
	Ile159Leu + Tyr161Asn
	Gly157Asp + Ile159Gly
	Ser158Asp + Ile159Gly
40	Gly152Pro + Gly155Glu
	Gly155Gln + Ser160Asp
	Gly152Asp + Tyr161Met
	Ser154Glu + Gly155Gln
	Gly155Glu + Tyr161Ala
45	Ala156Ser + Ser160Asp
	Ala156Glu + Tyr161Leu
	Gly152Pro + Gly157Asn
	Ser158Glu + Ile159Ser
	Gly157Asp + Tyr161Thr
50	Ser158Asp + Ile159Cys
	Gly155Pro + Tyr161Ala
	Gly152Asp + Tyr161Ala

	Ala156His + Ile159Asn
	Ser154Asp + Ala156His
	Gly157Pro + Ser158Asp
	Asn153Ser + Ile159Cys
5	Ser154Asp + Ala156Ser
	Ser154Glu + Tyr161Ala
	Ile159Leu + Tyr161Ala
	Gly152Glu + Gly155Gln
	Ala156Pro + Gly157Glu
10	Asn153Asp + Tyr161His
	Ala156Gln + Ile159Asn
	Gly152Pro + Ser154Asp
	Gly155Gln + Ala156Asp
	Ala156Ser + Ile159Gly
15	Asn153Asp + Gly157Gln
	Ile159Leu + Ser160Asp
	Gly155Glu + Ile159Gln
	Ser160Asp + Tyr161Thr
	Ser158Asp + Ile159Gln
20	Ser154Glu + Gly157Asn
	Ala156Gly + Ile159His
	Ala156Ser + Ser158Asp
	Asn153Gln + Gly155Glu
	Gly155Asn + Ser158Glu
25	Ser158Glu + Ile159Pro
	Ser158Asp + Ile159Leu

TABLE 17

## Loop 4 - Triple Mutation Variants

30	Ala156Asn + Gly157Asn + Tyr161Cys
	Gly152Gln + Gly155Glu + Tyr161Asn
	Gly152Gln + Asn153Gln + Ser154Glu
	Gly155Ser + Ser158Glu + Tyr161Gln
	Gly152Glu + Asn153Ser + Ala156Ser
35	Gly152Glu + Ile159Pro + Tyr161Asn
	Gly152Asp + Gly157Gln + Tyr161Met
	Gly155Asn + Ile159Thr + Tyr161His
	Asn153Ser + Gly155Ser + Ser158Glu
	Gly152Ser + Gly155Pro + Ser158Glu
40	Gly152Pro + Gly157Gln + Ser160Asp
	Gly152Gln + Ser154Asp + Tyr161Met
	Ala156Pro + Ile159Asn + Tyr161Ile
	Ile159Asn + Ser160Glu + Tyr161Ser
	Gly152Asp + Ile159Ala + Tyr161Cys
45	Asn153Gln + Ile159Ala + Ser160Glu
	Asn153Asp + Gly155Pro + Tyr161Asn
	Gly155Asn + Ala156Thr + Ser158Asp
	Gly152Gln + Ser154Asp + Tyr161Gln
	Gly157Ser + Ser158Asp + Tyr161Pro
50	Ser154Glu + Gly155Ser + Ile159Cys
	Gly155Gln + Ala156Asn + Ser158Asp
	Gly155Asn + Gly157Asn + Ile159Ala

	Gly157Asn + Ser158Glu + Ile159His
	Asn153Ser + Ile159Leu + Ser160Glu
	Ala156Gln + Gly157Asp + Tyr161Asn
	Gly155Asn + Ala156His + Tyr161Val
5	Asn153Gln + Ala156Asp + Ile159Gly
	Ala156His + Ser160Glu + Tyr161His
	Ala156Gln + Ser160Asp + Tyr161Ala
	Gly152Asp + Gly157Gln + Ile159Gly
	Asn153Glu + Ala156Pro + Ile159Ala
10	Gly152Gln + Gly155Asn + Ala156Thr
	Asn153Gln + Ile159Met + Ser160Glu
	Gly152Asp + Ala156Pro + Tyr161Cys
	Gly152Ser + Ser154Glu + Tyr161Val
	Gly155Asp + Gly157Ser + Tyr161Asn
15	Asn153Glu + Ser154Asp + Gly155Ser
	Asn153Glu + Ser154Asp + Ala156Pro
	Gly157Asn + Ser160Asp + Tyr161Asp
	Gly152Glu + Asn153Asp + Tyr161Met
	Gly152Asp + Asn153Asp + Ile159Leu
20	Ala156Asp + Gly157Glu + Tyr161Thr
	Asn153Ser + Ser154Glu + Gly155Asp
	Ser154Asp + Gly155Asp + Ala156Pro
	Asn153Ser + Ser154Asp + Gly155Glu
	Ser154Glu + Gly155Asp + Tyr161Ala
25	Gly157Asp + Ser158Glu + Tyr161Met
	Gly155Gln + Gly157Asp + Ser158Glu
	Ala156His + Gly157Glu + Ser158Asp
	Gly155Asp + Ala156Glu + Gly157Asp
	Ser154Asp + Ala156Glu + Tyr161Cys
30	Ser154Asp + Ala156Glu + Ile159Pro
	Ser154Asp + Ala156Glu + Ile159Asn
	Gly152Pro + Ala156Glu + Ser158Glu
	Gly155Asn + Ala156Glu + Ser158Glu
	Asn153Glu + Gly155Asp + Ile159Pro
35	Gly152Glu + Ile159Cys + Ser160Asp
	Gly152Glu + Ser154Glu + Gly157Gln
	Gly152Glu + Ser154Glu + Gly155Asn

TABLE 18

	Loop 4 - Quadruple Mutation Variants
40	Ala156Pro + Ile159Pro + Ser160Glu + Tyr161Thr
	Asn153Gln + Gly157Asn + Ile159Gln + Ser160Asp
	Asn153Gln + Ala156Gly + Gly157Glu + Ile159Asn
	Gly152Asn + Ile159Gln + Ser160Asp + Tyr161Met
45	Gly152Pro + Ser154Glu + Gly157Pro + Ile159Ala
	Ala156Ser + Gly157Ser + Ile159Gln + Tyr161Glu
	Asn153Ser + Gly157Ser + Ile159Ala + Ser160Asp
	Gly155Gln + Ser158Asp + Ile159Gly + Tyr161Met
	Gly152Ser + Ser154Asp + Ala156Gln + Ile159Cys
50	Ala156His + Gly157Pro + Ser158Glu + Tyr161Met
	Ala156Pro + Gly157Asn + Ile159Met + Ser160Asp
	Ser154Glu + Gly155Asn + Ile159Met + Tyr161Ala

	Gly152Ser + Ala156His + Gly157Pro + Ile159Gly
	Asn153Ser + Ile159Leu + Ser160Glu + Tyr161Thr
	Gly152Asn + Ser154Asp + Ala156Ser + Gly157Asn
	Ser154Asp + Gly155Gln + Ile159Leu + Tyr161Thr
5	Asn153Ser + Ser154Asp + Gly155Asn + Ile159Met
	Gly152Asn + Asn153Gln + Gly155Asn + Ile159Val
	Asn153Glu + Ser154Glu + Gly157Asn + Ile159Leu
	Asn153Glu + Ser154Asp + Gly155Pro + Ala156Ser
	Ser154Asp + Gly155Asp + Ala156Gly + Gly157Asn
10	Gly152Ser + Gly157Asp + Ser158Asp + Ile159Ser
	Gly155Pro + Gly157Asp + Ser158Asp + Ile159Pro
	Ala156Glu + Gly157Glu + Ser158Glu + Tyr161Ile
	Asn153Glu + Ser154Asp + Gly155Asp + Ala156Pro
	Ser154Glu + Ala156Glu + Gly157Ser + Ile159Ser
15	Ala156Glu + Gly157Asn + Ser158Asp + Ile159Asn
	Gly152Asn + Asn153Asp + Gly155Glu + Tyr161Gln
	Gly152Pro + Asn153Asp + Gly155Glu + Gly157Gln
	Gly152Asp + Ala156Pro + Ile159Val + Ser160Asp
	Gly152Asp + Asn153Gln + Ile159Ala + Ser160Asp
20	Gly152Glu + Ser154Glu + Ile159Gln + Tyr161Cys
	Gly152Glu + Ser154Asp + Gly157Pro + Tyr161Leu
	Gly155Asp + Ala156Asp + Gly157Pro + Ser158Asp
	Gly155Glu + Ala156Asp + Gly157Gln + Ser158Asp
	Gly152Asp + Asn153Glu + Ile159Thr + Ser160Glu
25	Gly152Glu + Asn153Asp + Ile159Leu + Ser160Glu
	Ala156Thr + Ser158Asp + Ser160Glu + Tyr161Cys
	Ser158Glu + Ile159Met + Ser160Asp + Tyr161Pro
	Ser158Glu + Ile159Cys + Ser160Glu + Tyr161Gly
	Gly155Pro + Ser158Glu + Ile159Gln + Ser160Asp
30	Gly157Pro + Ser158Asp + Ser160Glu + Tyr161Met
	Gly152Glu + Ser154Glu + Gly157Asn + Ser160Glu
	Gly152Glu + Ser154Asp + Gly157Gln + Ser160Asp
	Gly152Glu + Ser154Asp + Gly157Ser + Ser160Glu
	Gly152Glu + Ala156Gly + Ser158Asp + Ser160Glu
35	Gly152Asp + Ser158Asp + Ile159Leu + Ser160Glu
	Gly152Asp + Gly155Gln + Ser158Asp + Ser160Glu
	Ala156Glu + Ser158Glu + Ser160Asp + Tyr161Gly
	Gly157Asp + Ser158Glu + Ile159His + Ser160Glu
	Gly152Asn + Ser154Glu + Gly155Asp + Ser158Asp
40	Asn153Glu + Ser154Asp + Gly157Pro + Ser160Glu
	Asn153Asp + Ser154Asp + Ile159Ser + Ser160Glu
	Asn153Asp + Ser154Asp + Ser160Asp + Tyr161Ile
	Gly152Asn + Ser154Glu + Gly157Glu + Ser158Asp
	Gly152Glu + Ala156Asp + Ser158Glu + Ile159Val
45	Asn153Asp + Ser154Glu + Ala156Ser + Gly157Asp
	Asn153Glu + Ser154Glu + Gly155Ser + Gly157Asp
	Ser154Glu + Gly155Asp + Gly157Pro + Ser160Asp
	Gly152Asp + Asn153Glu + Ser158Glu + Ile159Ala

40

	Ala181Asp
	Ala181Gln
	Ala181Glu
	Ala181Gly
5	Ala181His
	Ala181Pro
	Ala181Ser
	Ala181Thr
	Ser182Asp
10	Ser182Glu
	Phe183Ala
	Phe183Asn
	Phe183Asp
	Phe183Cys
15	Phe183Gln
	Phe183Glu
	Phe183Gly
	Phe183His
	Phe183Ile
20	Phe183Leu
	Phe183Met
	Phe183Pro
	Phe183Ser
	Phe183Thr
25	Phe183Tyr
	Phe183Val
	Ser184Asp
	Ser184Glu
	Gln185Asn
30	Gln185Asp
	Gln185Glu
	Gln185Ser

TABLE 20

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35	Loop 5 - Double Mutation Variants
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	Ala181Asp + Phe183Gln
	Ser182Asp + Gln185Asn
	Phe183Met + Gln185Glu
	Ser182Glu + Gln185Asn
40	Ala181Pro + Ser182Glu
	Ala181Asn + Gln185Glu
	Ser182Glu + Phe183Leu
	Ala181Pro + Gln185Asp
	Phe183Ser + Gln185Glu
45	Ala181Gln + Ser182Glu
	Phe183Ile + Gln185Glu
	Ala181Ser + Gln185Asp
	Ala181Gln + Phe183Ser
	Ala181Thr + Phe183Asn
50	Ala181Gly + Gln185Asp
	Ala181His + Ser182Glu
	Phe183Gln + Gln185Ser

	Phe183Pro + Gln185Ser
	Ala181Asn + Ser182Asp
	Ala181Ser + Gln185Glu
	Ala181Asn + Phe183Asn
5	Ala181His + Phe183Asp
	Ala181Asn + Ser182Glu
	Ser182Asp + Phe183His
	Ala181Asp + Phe183Ile
	Phe183Leu + Gln185Asp
10	Ser182Glu + Phe183Ser
	Ala181His + Gln185Asp
	Phe183Val + Gln185Asp
	Ser182Asp + Phe183Thr
	Phe183Gly + Gln185Asp
15	Ala181Thr + Gln185Glu
	Ala181His + Gln185Glu
	Ser182Asp + Phe183Val
	Ala181Asp + Phe183Ser
	Ser182Asp + Phe183Cys
20	Ala181Pro + Phe183Asp
	Ala181Gly + Phe183Ala
	Ala181Pro + Phe183Asn
	Phe183Pro + Gln185Asp
	Ser182Glu + Phe183Tyr
25	Phe183Cys + Gln185Ser
	Ala181Thr + Ser182Glu
	Ala181Pro + Phe183Ala
	Ser182Glu + Phe183Thr
	Ala181Pro + Phe183Pro
30	Ser182Glu + Gln185Ser
	Ala181Gln + Ser182Asp
	Phe183Gly + Gln185Glu
	Ala181Gln + Gln185Asp
	Phe183Ser + Gln185Asp
35	Ser182Asp + Phe183Met
	Phe183Ala + Gln185Asn
	Ala181Asp + Phe183His
	Phe183Val + Gln185Glu
	Ala181Ser + Ser182Glu
40	Ala181Asn + Phe183Pro
	Phe183Tyr + Gln185Glu
	Ala181Glu + Phe183Cys
	Ser182Glu + Phe183Ile

TABLE 21

## Loop 5 - Triple Mutation Variants

	Ala181Glu + Phe183Val + Gln185Ser
	Ala181Pro + Ser182Asp + Gln185Asn
	Ala181Thr + Phe183His + Gln185Asp
	Ser182Glu + Phe183Ala + Gln185Asn
50	Ala181Thr + Ser182Asp + Gln185Asn
	Ala181Gln + Ser182Asp + Phe183Ala

	Ser182Asp + Phe183Asn + Gln185Asn
	Ala181Gln + Phe183Gln + Gln185Glu
	Ser182Asp + Phe183Met + Gln185Asn
	Ala181Pro + Ser182Glu + Phe183Gly
5	Ala181His + Phe183Ala + Gln185Ser
	Ala181Ser + Ser182Glu + Gln185Asn
	Ala181Gly + Phe183Ile + Gln185Glu
	Ala181Asn + Phe183Tyr + Gln185Asn
	Ser182Asp + Phe183Leu + Gln185Asn
10	Ser182Asp + Phe183Val + Gln185Asn
	Ala181Glu + Phe183Leu + Gln185Ser
	Ala181Glu + Phe183Tyr + Gln185Ser
	Ala181Gln + Ser182Asp + Phe183Gly
	Ala181Thr + Phe183Ile + Gln185Ser
15	Ala181Gln + Ser182Glu + Gln185Ser
	Ala181Gln + Ser182Glu + Phe183His
	Ala181Asp + Phe183Ala + Gln185Asn
	Ala181Thr + Ser182Asp + Phe183Thr
	Ala181Ser + Phe183Pro + Gln185Glu
20	Ser182Glu + Phe183Ala + Gln185Ser
	Ala181Asn + Phe183Cys + Gln185Ser
	Ala181Pro + Phe183Thr + Gln185Asp
	Ala181Thr + Phe183Val + Gln185Asn
	Ala181Gln + Ser182Asp + Gln185Ser
25	Ala181Asn + Ser182Asp + Gln185Ser
	Ser182Asp + Phe183Thr + Gln185Ser
	Ala181Ser + Phe183Asp + Gln185Asn
	Ser182Asp + Phe183Thr + Gln185Asn
	Ala181Gly + Phe183Asn + Gln185Ser
30	Ala181Asp + Phe183Met + Gln185Ser
	Ala181Asp + Phe183Pro + Gln185Asn
	Ala181Gly + Ser182Asp + Phe183Gly
	Ala181Gln + Ser182Glu + Gln185Asn
	Ala181Thr + Ser182Asp + Gln185Ser
35	Ala181Asn + Phe183Asn + Gln185Asp
	Ala181Thr + Phe183Asp + Gln185Ser
	Ala181Gln + Phe183Ser + Gln185Ser
	Ser182Asp + Phe183Gly + Gln185Ser
	Ala181Gly + Ser182Glu + Phe183Leu
40	Ala181Asn + Ser182Glu + Gln185Asn
	Ala181Glu + Ser182Glu + Phe183Ser
	Ala181Glu + Ser182Glu + Phe183Gly
	Ala181Glu + Ser182Glu + Phe183Leu
45	Ala181Glu + Ser182Glu + Phe183Val
	Ala181Glu + Ser182Asp + Phe183Met
	Ala181Asp + Ser182Asp + Phe183Met
	Ala181Asp + Ser182Asp + Phe183Cys
	Ala181Glu + Ser182Asp + Gln185Asn
	Ala181Asp + Ser182Asp + Gln185Asn
50	Ala181Glu + Ser182Glu + Phe183Asn
	Ala181Asp + Ser182Glu + Phe183Ser
	Ala181Glu + Ser182Asp + Gln185Ser
	Ala181Glu + Ser182Glu + Phe183Thr
	Ala181Glu + Ser182Asp + Phe183Leu

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TABLE 22

Loop 5 - Quadruple Mutation Variants	
5	Ala181Ser + Ser182Asp + Phe183Ala + Gln185Ser
	Ala181Ser + Ser182Glu + Phe183Pro + Gln185Ser
	Ala181Gly + Ser182Asp + Phe183Gly + Gln185Asn
	Ala181Gly + Ser182Glu + Phe183Pro + Gln185Ser
	Ala181Thr + Ser182Glu + Phe183Thr + Gln185Ser
10	Ala181Gly + Ser182Glu + Phe183Thr + Gln185Ser
	Ala181His + Ser182Glu + Phe183His + Gln185Asn
	Ala181Gly + Ser182Glu + Phe183Ser + Gln185Ser
	Ala181Gln + Ser182Glu + Phe183His + Gln185Asn
	Ala181Asn + Ser182Glu + Phe183Cys + Gln185Ser
15	Ala181Pro + Ser182Glu + Phe183Met + Gln185Ser
	Ala181Gln + Ser182Asp + Phe183Gln + Gln185Asn
	Ala181Thr + Ser182Asp + Phe183His + Gln185Asn
	Ala181Gly + Ser182Glu + Phe183Leu + Gln185Ser
	Ala181Asn + Ser182Asp + Phe183Ile + Gln185Ser
20	Ala181Gly + Ser182Asp + Phe183Ala + Gln185Ser
	Ala181Asn + Ser182Asp + Phe183Ser + Gln185Asn
	Ala181Asn + Ser182Glu + Phe183Gly + Gln185Ser
	Ala181Asn + Ser182Glu + Phe183Met + Gln185Asn
	Ala181His + Ser182Glu + Phe183Asn + Gln185Asn
25	Ala181Gly + Ser182Glu + Phe183Tyr + Gln185Asn
	Ala181Asn + Ser182Asp + Phe183Asn + Gln185Asn
	Ala181Glu + Ser182Glu + Phe183Asn + Gln185Asn
	Ala181Asp + Ser182Glu + Phe183Ile + Gln185Asn
	Ala181Glu + Ser182Asp + Phe183Gln + Gln185Asn
30	Ala181Asp + Ser182Asp + Phe183His + Gln185Asn
	Ala181Glu + Ser182Asp + Phe183Ser + Gln185Asn
	Ala181Glu + Ser182Asp + Phe183Leu + Gln185Asn
	Ala181Glu + Ser182Asp + Phe183Leu + Gln185Ser
	Ala181Asp + Ser182Glu + Phe183Thr + Gln185Ser
35	Ala181Asp + Ser182Asp + Phe183Gln + Gln185Asn
	Ala181Asp + Ser182Asp + Phe183Ala + Gln185Asn
	Ala181Asp + Ser182Glu + Phe183Ala + Gln185Asn
	Ala181Glu + Ser182Asp + Phe183Met + Gln185Ser
	Ala181Glu + Ser182Glu + Phe183Thr + Gln185Asn
40	Ala181Asp + Ser182Asp + Phe183Gly + Gln185Ser
	Ala181Glu + Ser182Asp + Phe183Ala + Gln185Asn
	Ala181His + Ser182Glu + Phe183Glu + Gln185Asn
	Ala181Ser + Ser182Asp + Phe183Glu + Gln185Ser
	Ala181Ser + Ser182Asp + Phe183Asp + Gln185Ser
45	Ala181Pro + Ser182Glu + Phe183Asp + Gln185Ser
	Ala181Asp + Ser182Asp + Phe183Asp + Gln185Asn
	Ala181Glu + Ser182Asp + Phe183Glu + Gln185Ser
	Ala181Ser + Ser182Asp + Phe183Glu + Gln185Glu
	Ala181Gly + Ser182Glu + Phe183Glu + Gln185Glu
50	Ala181Gln + Ser182Glu + Phe183Asp + Gln185Asp
	Ala181Ser + Ser182Asp + Phe183Asp + Gln185Asp
	Ala181Thr + Ser182Asp + Phe183Asp + Gln185Asp
	Ala181Pro + Ser182Asp + Phe183Glu + Gln185Asp

	Ala181Asn + Ser182Glu + Phe183Asp + Gln185Asp
	Ala181Thr + Ser182Asp + Phe183Asp + Gln185Glu
	Ala181Pro + Ser182Glu + Phe183Glu + Gln185Glu
	Ala181Asn + Ser182Asp + Phe183Asp + Gln185Glu
5	Ala181Thr + Ser182Glu + Phe183Asp + Gln185Asp
	Ala181His + Ser182Glu + Phe183Glu + Gln185Glu
	Ala181Glu + Ser182Glu + Phe183Ser + Gln185Glu
	Ala181Glu + Ser182Glu + Phe183Val + Gln185Glu
	Ala181Asp + Ser182Glu + Phe183Pro + Gln185Glu
10	Ala181Glu + Ser182Glu + Phe183Thr + Gln185Asp
	Ala181Asp + Ser182Asp + Phe183Cys + Gln185Glu

TABLE 23

## Loop 6 - Single Mutation Variants

15	Val193Ala
	Val193Asn
	Val193Asp
	Val193Cys
	Val193Gln
20	Val193Glu
	Val193Gly
	Val193His
	Val193Met
	Val193Pro
25	Val193Ser
	Val193Thr
	Ala194Asn
	Ala194Asp
	Ala194Gln
30	Ala194Glu
	Ala194Gly
	Ala194His
	Ala194Pro
	Ala194Ser
35	Ala194Thr
	Pro195Asn
	Pro195Asp
	Pro195Gln
	Pro195Glu
40	Pro195Gly
	Pro195Ser
	Gly196Asn
	Gly196Asp
	Gly196Gln
45	Gly196Glu
	Gly196Pro
	Gly196Ser
	Val197Ala
	Val197Asp
50	Val197Cys
	Val197Gln
	Val197Glu

	Val197Gly
	Val197His
	Val197Met
	Val197Pro
5	Val197Ser
	Val199Ala
	Val199Asn
	Val199Asp
	Val199Cys
10	Val199Gln
	Val199Glu
	Val199Gly
	Val199His
	Val199Met
15	Val199Pro
	Val199Ser
	Val199Thr
	Gln200Asn
	Gln200Ser
20	Ser201Asp
	Ser201Glu
	Thr202Asn
	Thr202Asp
	Thr202Gln
25	Thr202Glu
	Thr202Gly
	Thr202Pro
	Thr202Ser
	Tyr203Ala
30	Tyr203Asn
	Tyr203Asp
	Tyr203Gln
	Tyr203His
	Tyr203Ile
35	Tyr203Met
	Tyr203Pro
	Tyr203Ser
	Pro204Asn
	Pro204Asp
40	Pro204Gln
	Pro204Glu
	Pro204Gly
	Pro204Ser
	Gly205Asn
45	Gly205Asp
	Gly205Gln
	Gly205Glu
	Gly205Pro
	Gly205Ser
50	Ser206Asp
	Ser206Glu
	Thr207Asn
	Thr207Gln
	Thr207Glu

	Thr207Gly
	Thr207Pro
	Thr207Ser
	Tyr208Ala
5	Tyr208Asn
	Tyr208Asp
	Tyr208Cys
	Tyr208Gln
	Tyr208Glu
10	Tyr208Gly
	Tyr208His
	Tyr208Ile
	Tyr208Leu
	Tyr208Met
15	Tyr208Pro
	Tyr208Val
	Ala209Asn
	Ala209Asp
	Ala209Gln
20	Ala209Glu
	Ala209Gly
	Ala209His
	Ala209Pro
	Ala209Ser
25	Ala209Thr
	Ser210Asp
	Ser210Glu
	Leu211Ala
	Leu211Asn
30	Leu211Asp
	Leu211Cys
	Leu211Gln
	Leu211Gly
	Leu211His
35	Leu211Ile
	Leu211Met
	Leu211Pro
	Leu211Ser
	Leu211Thr
40	Leu211Val
	Asn212Glu
	Gly213Asn
	Gly213Asp
	Gly213Gln
45	Gly213Glu
	Gly213Pro
	Gly213Ser
	Thr214Asn
	Thr214Asp
50	Thr214Gln
	Thr214Glu
	Thr214Gly
	Thr214Pro
	Thr214Ser

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TABLE 24

Loop 6 - Double Mutation Variants	
5	Gly196Asn + Ala209Asp
	Val199Ser + Tyr208Asn
	Pro195Asn + Thr207Asp
	Val197Ala + Asn212Asp
	Asn198Gln + Thr207Ser
10	Val199Gly + Tyr208Ile
	Gly196Asn + Ala209Pro
	Val199Gly + Gln200Ser
	Pro195Ser + Gly205Gln
	Val199Ser + Leu211Asp
15	Gly196Gln + Val197Asn
	Thr202Asn + Thr214Glu
	Ala194Thr + Thr202Pro
	Val199Asn + Ser210Glu
	Thr202Ser + Asn212Gln
20	Ser210Asp + Thr214Asn
	Pro195Gly + Asn212Asp
	Asn198Gln + Tyr208Gln
	Val197Asn + Thr214Glu
	Tyr208Asp + Leu211Gln
25	Thr202Ser + Ser210Glu
	Val197Cys + Ser210Glu
	Gln200Glu + Tyr203Gly
	Ala209Thr + Leu211Glu
	Val197Ser + Gln200Glu
30	Thr202Gly + Ser210Glu
	Gln200Ser + Gly213Pro
	Gly196Ser + Thr207Gly
	Pro204Gln + Ser210Glu
	Val199Cys + Pro204Gly
35	Gly213Pro + Thr214Pro
	Gly196Asn + Ser210Glu
	Thr207Gln + Ser210Glu
	Val199Met + Gln200Asp
	Thr202Ser + Tyr203Ile
40	Pro195Ser + Asn212Ser
	Val197Glu + Tyr208Gln
	Asn198Glu + Leu211Cys
	Gly196Gln + Ser206Glu
	Ala194Ser + Thr214Ser
45	Val197Ser + Tyr203Ala
	Ser210Asp + Gly213Pro
	Tyr203His + Gly213Asp
	Val197Ser + Val199Ser
	Ala209Pro + Ser210Asp
50	Thr207Asp + Thr214Ser
	Thr207Gly + Ala209His
	Pro195Asn + Asn198Asp
	Val197Pro + Ser206Asp

	Gln200Ser + Asn212Asp
	Val197Thr + Asn212Asp
	Gln200Asn + Thr214Glu
	Ala209Asp + Thr214Ser
5	Ala209Asn + Asn212Ser
	Ala194Ser + Asn212Glu
	Val197Glu + Gly213Asn
	Tyr203Cys + Asn212Asp
	Pro195Gln + Val197Cys
10	Asn198Ser + Tyr208Ala
	Gly205Gln + Ala209Glu
	Gly205Ser + Leu211Ser
	Gly205Gln + Tyr208Ala
	Ala194Ser + Asn198Asp
15	Ala194Thr + Pro195Ser
	Val199Thr + Pro204Glu
	Thr202Gln + Ser210Asp
	Ser206Glu + Leu211His
	Asn198Glu + Thr202Asn
20	Ser210Asp + Gly213Ser
	Gly196Ser + Thr202Gln
	Pro204Gln + Gly213Asp
	Asn198Glu + Pro204Gly
	Thr202Gly + Tyr208Met
25	Pro195Gly + Gly213Glu
	Pro195Ser + Pro204Asp
	Pro195Ser + Tyr203Thr
	Ala194Asn + Tyr203Pro
	Ala194Pro + Gln200Asp
30	Pro204Asp + Gly205Asn
	Gly196Pro + Asn198Gln
	Thr207Ser + Leu211Gly
	Pro195Ser + Leu211Ile
	Pro204Glu + Leu211Gly
35	Val199Ser + Tyr208Ala
	Gly196Ser + Ser210Asp
	Ala194Thr + Leu211Gly
	Thr207Ser + Ser210Glu
	Gly196Pro + Thr207Glu
40	Pro204Glu + Leu211Met
	Pro195Asn + Asn198Ser
	Tyr203Met + Thr207Gln
	Pro204Gly + Ala209Glu
	Val197Glu + Asn212Ser
45	Pro195Ser + Ala209Pro
	Gly196Ser + Leu211Gly
	Gly205Pro + Leu211Met
	Val197Gln + Pro204Ser
	Val199Asn + Ser206Asp
50	Val197Asp + Thr202Asn
	Gly196Gln + Thr214Gly
	Pro204Asn + Asn212Gln
	Val199Asn + Gln200Asn
	Pro195Gly + Leu211Gly

	Tyr203Cys + Pro204Ser
	Gln200Asp + Leu211Cys
	Asn198Gln + Ser210Glu
	Ala194Gly + Gln200Glu
5	Gly196Gln + Pro204Glu
	Val197Pro + Thr202Asn
	Ala194Gly + Val199Ala
	Ala194Thr + Leu211Asn
	Asn198Gln + Gly205Asp
10	Tyr203Asn + Leu211His
	Asn212Asp + Gly213Gln
	Asn198Gln + Ser206Asp
	Val199Thr + Gln200Glu
	Thr202Ser + Asn212Asp
15	Ala194Gly + Ser210Glu
	Ser206Glu + Ala209Asn
	Ala209Asp + Asn212Ser
	Val199Gly + Asn212Ser
	Ala194Pro + Gly213Asp
20	Gln200Asp + Pro204Gly
	Val197Thr + Tyr203Ala
	Asn198Gln + Leu211Pro
	Pro195Gln + Leu211Gln
	Gly196Gln + Thr214Glu
25	Pro195Asn + Ser210Glu
	Thr207Asn + Leu211Asp
	Ser210Glu + Gly213Pro
	Gly205Pro + Thr207Asp
	Gln200Glu + Thr202Gln
30	Thr202Ser + Gly213Asp
	Pro204Gln + Thr214Gln
	Gly196Ser + Gln200Asp
	Val197Ser + Gln200Asp
	Thr202Pro + Thr207Asp
35	Tyr203Thr + Tyr208His
	Pro195Gln + Thr202Asn
	Val197Asn + Asn212Asp
	Val197Met + Thr202Gln
	Val199Met + Thr202Pro
40	Ala209Glu + Leu211Met
	Gly196Asn + Val199His
	Ala194Gly + Ser206Glu
	Gly196Asn + Pro204Asn
	Gly196Asn + Gln200Glu
45	Val197Ser + Ser210Asp
	Pro195Gln + Pro204Gln
	Ala194His + Asn198Gln
	Val197Cys + Asn212Gln
	Asn198Glu + Ala209Ser
50	Ala194Gln + Val199Pro
	Asn198Asp + Val199Gln
	Asn198Asp + Thr214Pro
	Pro204Asn + Tyr208Asp
	Tyr203Ser + Tyr208Leu

50

	Gly196Gln + Ala209Asp
	Ser206Glu + Asn212Gln
	Thr207Ser + Ala209His
	Val197Thr + Ser210Asp
5	Tyr208Gln + Ser210Asp
	Asn198Ser + Ser210Glu
	Gly196Ser + Thr214Asp
	Ala194Gln + Thr202Asn
	Pro195Gly + Thr214Asn
10	Leu211Thr + Thr214Asp
	Pro195Ser + Asn198Glu
	Gly196Ser + Gly205Ser
	Ala194His + Thr202Asn
	Thr207Gly + Tyr208Met
15	Ala194Ser + Val197His

TABLE 25

## Loop 6 - Triple Mutation Variants

	Ala194Thr + Ser206Glu + Tyr208Cys
20	Gly196Asn + Tyr203Pro + Ala209Gly
	Pro195Gly + Asn198Ser + Thr207Glu
	Pro195Gly + Gly205Gln + Gly213Ser
	Tyr203Leu + Pro204Gln + Gly205Gln
	Gly196Gln + Val197Asn + Ser206Asp
25	Leu211Ser + Gly213Ser + Thr214Gln
	Pro195Asn + Pro204Glu + Gly205Gln
	Asn198Asp + Thr202Gly + Gly213Gln
	Tyr203Ala + Leu211His + Thr214Gln
	Ala194Thr + Val197Ser + Val199Gly
30	Ala194Gln + Thr207Asp + Thr214Ser
	Gly196Asn + Pro204Gln + Ala209Asn
	Thr202Pro + Tyr208Asp + Leu211Thr
	Val197Gly + Ala209Gln + Asn212Gln
	Gly196Ser + Gly205Asp + Leu211Ile
35	Ala194Ser + Val197Pro + Asn198Asp
	Gly196Asn + Asn198Gln + Leu211Ser
	Ala194Thr + Ala209His + Thr214Asp
	Ala194Pro + Pro195Ser + Asn212Asp
	Thr202Ser + Tyr203Gln + Ser210Asp
40	Thr202Ser + Tyr203Leu + Thr214Glu
	Ala194Ser + Pro195Gly + Leu211Val
	Ala194Pro + Thr207Glu + Leu211Ala
	Gly196Asn + Ser206Glu + Leu211Gly
	Gly196Ser + Gly205Ser + Ser210Asp
45	Ala194Thr + Val197Gly + Leu211Ser
	Pro204Glu + Ala209His + Thr214Pro
	Gly196Ser + Gly205Asp + Leu211His
	Val197Cys + Thr202Gly + Thr207Gln
	Asn198Ser + Pro204Gly + Gly213Asp
50	Ala194Gly + Ala209Pro + Ser210Asp
	Gly196Pro + Tyr203Val + Tyr208Cys
	Val199Ala + Thr207Asn + Tyr208Val



5 Tyr203Cys + Ser210Asp + Leu211Pro  
Ala194Ser + Val197Ser + Gln200Asp  
Ala194Asn + Pro195Gly + Leu211Ile  
Val199Ser + Leu211Asn + Gly213Asp  
Ala194Asn + Ser206Glu + Thr214Gly  
Ala194Gly + Pro195Gly + Val197Asp  
Pro195Gln + Ala209Ser + Ser210Asp  
Val197Thr + Asn198Gln + Tyr203Leu  
Val197Ala + Thr202Gln + Ser206Glu  
10 Gly196Pro + Pro204Gly + Asn212Glu  
Thr202Gly + Pro204Glu + Tyr208Ser  
Gln200Ser + Thr202Pro + Pro204Ser  
Gln200Glu + Tyr203Thr + Pro204Asn  
Asn198Asp + Val199Gln + Pro204Asn  
15 Ala194Pro + Pro195Gln + Val199Gly  
Pro195Asn + Pro204Asn + Ser206Glu  
Thr202Asn + Leu211Thr + Asn212Gln  
Gly196Gln + Thr202Pro + Gly213Asp  
Asn198Glu + Gln200Asn + Leu211Gly  
Val199Gly + Thr202Gln + Gly213Glu  
20 Tyr203Ser + Thr207Pro + Ser210Asp  
Pro195Gly + Gly196Gln + Val197Glu  
Pro195Gly + Thr207Gly + Tyr208Ser  
Thr202Gly + Leu211Glu + Gly213Gln  
Val197Gly + Thr202Pro + Asn212Glu  
25 Gly196Gln + Gly205Gln + Ser206Glu  
Pro195Gly + Val199Thr + Thr214Glu  
Gly196Ser + Gln200Ser + Leu211Asp  
Gly196Asn + Tyr208Glu + Thr214Pro  
30 Tyr203Thr + Pro204Asn + Ser206Glu  
Val199Thr + Thr202Asn + Tyr208Gly  
Thr202Pro + Tyr203Thr + Pro204Glu  
Gly196Gln + Asn212Asp + Thr214Gln  
Pro195Gly + Thr202Gly + Asn212Ser  
35 Pro195Gly + Tyr208Gln + Ser210Asp  
Ala194Pro + Ser206Glu + Leu211Ser  
Ala194His + Pro204Asn + Gly213Gln  
Gly196Ser + Tyr208Met + Ser210Asp  
Val197Glu + Val199Ser + Tyr203Asn  
40 Thr202Gly + Ala209Asn + Asn212Asp  
Val197Ser + Ser210Asp + Gly213Pro  
Ala194Ser + Tyr208Ile + Asn212Glu  
Ala194Gly + Asn198Gln + Ala209His  
Ala194Ser + Thr207Pro + Gly213Glu  
45 Val199Thr + Leu211Asp + Asn212Ser  
Val199Pro + Thr202Ser + Leu211Asn  
Ser206Glu + Tyr208Cys + Thr214Asn  
Ala209Ser + Leu211Ile + Asn212Glu  
Asn198Glu + Tyr208Ala + Ala209Ser  
50 Ala194Pro + Asn198Asp + Gly213Gln  
Ala194Asn + Pro195Ser + Leu211Glu  
Gly196Pro + Pro204Gly + Gly205Pro  
Tyr203Ile + Tyr208Thr + Ala209Glu  
Gly196Gln + Thr202Gln + Asn212Asp

5      Ala194Asn + Val197Pro + Thr202Pro  
Ala194Gln + Val199Gly + Ala209Asp  
Ala194Ser + Pro195Gly + Asn212Gln  
Pro195Gln + Tyr203Pro + Ser210Asp  
Val197Thr + Thr202Gln + Tyr208Asn  
Ala194Thr + Gly196Ser + Leu211Asn  
Ala194Gly + Thr202Ser + Pro204Asp  
Ala194Gly + Gly205Glu + Gly213Ser  
Val199Pro + Thr207Pro + Tyr208Met  
10      Ala194Pro + Asn198Glu + Ala209His  
Tyr208Leu + Ser210Glu + Thr214Pro  
Gly196Asn + Val199Ala + Gly205Asp  
Gly196Pro + Gly205Glu + Tyr208Ser  
Pro195Asn + Gly196Gln + Val197Cys  
15      Asn198Ser + Gln200Glu + Leu211Asn  
Thr202Ser + Tyr203Cys + Thr214Gln  
Ala194Ser + Gly196Gln + Thr207Asp  
Gly196Ser + Gly205Glu + Tyr208Ile  
Tyr203Met + Gly205Asn + Gly213Pro  
Pro204Gln + Gly205Asp + Ala209His  
20      Gly205Asn + Leu211Asp + Thr214Ser  
Tyr203Met + Ala209His + Leu211Met  
Val199Asn + Thr202Ser + Gly213Glu  
Val197Thr + Pro204Asp + Asn212Gln  
Ala194Gly + Ser210Glu + Leu211Pro  
25      Val197His + Ala209Gly + Ser210Asp  
Gln200Glu + Thr202Gln + Tyr208His  
Ala194Ser + Ser206Asp + Leu211Ala  
Pro195Ser + Asn198Asp + Gly205Pro  
Tyr203Pro + Thr207Asp + Thr214Pro  
30      Pro195Ser + Val199His + Leu211Pro  
Tyr203Met + Thr207Glu + Ala209His  
Val197Cys + Gln200Asn + Ser206Glu  
Val197Glu + Thr202Gln + Tyr208His  
Pro195Gln + Val197Cys + Pro204Gly  
35      Val199Pro + Gly205Ser + Gly213Asp  
Ala194Thr + Thr207Ser + Ala209Glu  
Val197Met + Gly205Pro + Tyr208Gly  
Gly196Ser + Gly205Glu + Ala209Thr  
Pro195Ser + Val199Thr + Gly205Ser  
40      Ala194Ser + Asn198Asp + Gly213Ser  
Asn198Glu + Val199Ser + Thr207Pro  
Ala194Thr + Gly196Pro + Ser210Glu  
Gly196Asn + Thr202Pro + Leu211Met  
Tyr203Met + Asn212Asp + Thr214Ser  
45      Tyr203Gly + Ala209Ser + Ser210Asp  
Ala194Ser + Thr207Ser + Leu211Pro  
Tyr208Cys + Gly213Asn + Thr214Pro  
Val197Met + Pro204Glu + Gly213Ser  
Gln200Ser + Tyr203Ala + Pro204Asp  
50      Pro195Gly + Ser206Asp + Leu211Ile  
Tyr203Gly + Tyr208Asp + Leu211Asn  
Val197Thr + Ser206Glu + Thr214Pro  
Tyr203Ala + Pro204Asn + Gly213Glu

	Tyr208Ile + Ala209Gly + Leu211Gln
	Asn198Ser + Pro204Gly + Asn212Glu
	Ala194Gln + Val197Cys + Pro204Asn
	Ala209Thr + Leu211Ser + Gly213Pro
5	Gly196Ser + Thr202Pro + Asn212Asp
	Val197His + Tyr203Gln + Tyr208Cys
	Val199Ser + Leu211Thr + Gly213Pro
	Pro195Asn + Val197Asn + Thr202Pro
	Val199Pro + Pro204Gly + Gly205Asn
10	Pro195Gly + Asn198Asp + Thr214Gly
	Gln200Glu + Tyr203Ser + Asn212Gln
	Pro195Gly + Val199Gln + Thr214Pro
	Thr202Ser + Pro204Ser + Gly213Glu
	Pro195Gly + Thr202Pro + Gly205Glu
15	Thr202Gln + Tyr203Gly + Ala209Asn
	Pro195Asn + Gly196Gln + Asn212Asp
	Thr202Gly + Tyr208Ile + Leu211Val
	Asn198Glu + Tyr203Cys + Pro204Gly
	Ala194Ser + Thr207Ser + Ser210Asp
20	Gln200Asp + Thr202Pro + Tyr203Ala
	Val199Cys + Ala209Gln + Ser210Asp
	Ala194Asn + Val199Cys + Leu211Pro
	Gln200Ser + Ser210Glu + Leu211Asn
	Tyr203Gln + Thr207Glu + Tyr208Ser
25	Ala194Thr + Thr202Asn + Thr207Gly
	Pro195Gly + Gly196Gln + Ser210Asp
	Gln200Ser + Pro204Gly + Ser206Glu
	Ala194Asn + Gly196Gln + Asn198Glu
	Val197Thr + Pro204Gln + Gly205Glu
30	Val199Ala + Gln200Asp + Gly213Asn
	Val197Met + Tyr203Val + Gly213Pro
	Ala194Gln + Tyr203Val + Thr214Glu
	Val199Cys + Ala209His + Leu211Met
	Thr202Gln + Tyr203Ile + Ala209Gln
35	Ala194Gly + Ser206Glu + Leu211Gly
	Gly196Pro + Tyr208Gly + Ser210Glu
	Val197Gln + Thr202Gly + Gly205Asp
	Val197Pro + Gly205Asp + Thr214Pro

TABLE 26

40

## Loop 6 - Quadruple Mutation Variants

45

50

	Pro195Asn + Val197Ala + Thr207Ser + Asn212Asp
	Asn198Gln + Val199Gly + Tyr208Ile + Asn212Asp
	Tyr203His + Leu211Pro + Asn212Gln + Gly213Gln
	Ala194His + Asn198Ser + Thr202Asn + Ala209Asp
	Gly196Gln + Asn198Ser + Thr202Ser + Pro204Glu
	Pro195Gln + Asn198Gln + Gly205Glu + Ala209Pro
	Val199His + Pro204Asp + Gly205Pro + Leu211Gly
	Gly196Gln + Gly205Asn + Ser206Asp + Thr214Ser
	Gln200Glu + Thr202Pro + Leu211Asn + Gly213Asn
	Gly196Gln + Pro204Asn + Asn212Gln + Thr214Gly
	Pro195Gly + Val199Asn + Gln200Asn + Leu211Gly

5 Pro195Gln + Pro204Ser + Gly205Glu + Tyr208Ile  
Val199Ala + Tyr203Gln + Thr207Gly + Ser210Glu  
Ala194His + Val199Ser + Pro204Asn + Gly205Glu  
Gly196Pro + Asn198Asp + Gln200Asn + Ala209Pro  
Pro195Gln + Gln200Asp + Tyr203Asn + Leu211Cys  
Pro195Ser + Thr202Pro + Asn212Asp + Thr214Ser  
Ala194Gly + Val197Glu + Tyr203Leu + Leu211Cys  
Asn198Gln + Thr202Pro + Tyr208Met + Thr214Glu  
Gly196Ser + Val199Ser + Gly205Gln + Ser210Glu  
10 Tyr203Ala + Gly205Gln + Ser206Asp + Leu211Gln  
Gly196Ser + Val199His + Gln200Asp + Thr207Ser  
Pro195Gln + Gly196Gln + Gln200Glu + Tyr203Ser  
Gly196Ser + Gln200Glu + Gly205Gln + Gly213Asn  
Pro195Ser + Gly196Asn + Ser206Glu + Thr214Gly  
15 Gly196Asn + Val199Asn + Ser206Glu + Tyr208Pro  
Val197Gln + Gly205Ser + Ser210Glu + Thr214Ser  
Pro195Asn + Gln200Glu + Gly205Asn + Leu211Pro  
Tyr203Pro + Tyr208His + Leu211Val + Thr214Pro  
Ala194His + Val199Ser + Thr202Gly + Tyr208Asp  
20 Pro195Asn + Val199Asn + Ser206Asp + Gly213Ser  
Ala194Ser + Pro195Asn + Val199Ala + Gly205Glu  
Ala194Thr + Gly196Gln + Val199Gly + Tyr203Ile  
Val199Ala + Ala209Asp + Leu211Val + Asn212Gln  
Val197Gly + Asn198Ser + Tyr203Leu + Ser210Asp  
25 Gln200Ser + Thr207Gly + Tyr208Thr + Ser210Asp  
Pro204Gly + Gly205Asp + Thr207Asn + Tyr208Leu  
Ala194Gln + Thr202Ser + Ala209Ser + Asn212Glu  
Val197Met + Thr202Gly + Pro204Gly + Thr214Glu  
Val199Ala + Pro204Gln + Gly205Asp + Tyr208Gly  
30 Asn198Glu + Tyr208Cys + Ala209Gln + Leu211Val  
Gln200Ser + Pro204Ser + Ala209Asn + Leu211Glu  
Pro195Ser + Val197Thr + Gly205Gln + Thr214Pro  
Pro195Asn + Thr202Asn + Gly205Asp + Tyr208Ser  
Pro195Gly + Tyr203Pro + Leu211Ala + Asn212Glu  
35 Thr202Gly + Pro204Asn + Gly205Asn + Gly213Gln  
Val197Gly + Gln200Glu + Tyr208Ala + Asn212Ser  
Ala194Ser + Pro195Ser + Tyr203Ala + Ala209Asp  
Pro195Gln + Val197Thr + Val199Pro + Ala209His  
Gly196Asn + Ser206Asp + Leu211His + Thr214Ser  
40 Val197Asp + Tyr203His + Tyr208Cys + Thr214Gly  
Ala194Gln + Pro195Asn + Ala209His + Ser210Asp  
Val199Ser + Gln200Ser + Tyr203Asn + Ser206Asp  
Ala194Asn + Pro195Gln + Ser206Glu + Gly213Ser  
Pro195Ser + Tyr203Met + Ser206Asp + Gly213Gln  
45 Ala194Gly + Val199Thr + Tyr208His + Gly213Pro  
Ala194Pro + Val197Cys + Gln200Asp + Pro204Gly  
Thr202Gln + Thr207Glu + Tyr208Cys + Leu211Pro  
Gly196Gln + Asn198Glu + Tyr203Gln + Gly205Pro  
Thr202Asn + Tyr203Pro + Gly205Ser + Gly213Glu  
50 Pro195Asn + Asn198Ser + Ala209Gly + Leu211Thr  
Asn198Asp + Gln200Ser + Tyr203Pro + Gly205Asn  
Pro195Gln + Val199Gly + Tyr203Asn + Thr207Glu  
Ala194Gln + Thr202Gln + Tyr203His + Tyr208His  
Ala194Ser + Pro204Asp + Thr207Pro + Tyr208Thr

	Asn198Gln + Val199Gln + Gln200Asn + Ala209Gln
	Ala194Gly + Asn198Ser + Pro204Asp + Ala209His
	Gly196Gln + Val197Thr + Ser206Glu + Ala209Pro
	Gln200Ser + Thr202Pro + Tyr203Thr + Thr207Gly
5	Ala194Thr + Val197Gly + Gln200Asn + Thr207Asp
	Val197Pro + Val199Asn + Gln200Glu + Leu211Ile
	Gly196Pro + Pro204Ser + Ala209Ser + Leu211Gln
	Pro195Gly + Gly196Gln + Asn198Gln + Tyr208Glu
	Gln200Ser + Tyr203Met + Gly205Glu + Tyr208Asn
10	Ala194Gln + Pro195Gly + Ala209Gly + Ser210Glu
	Gly196Asn + Val197Asn + Ser210Asp + Asn212Gln
	Gly196Gln + Thr202Ser + Gly205Ser + Ser210Glu
	Gln200Asn + Gly205Pro + Leu211Cys + Thr214Pro
	Ala194Ser + Gly196Gln + Thr207Ser + Ser210Asp
15	Val197Gln + Thr202Pro + Tyr203Ala + Ser206Asp
	Gly196Gln + Asn198Glu + Asn212Ser + Gly213Asn
	Pro195Asn + Val197Gln + Gly205Gln + Leu211Glu
	Thr207Glu + Tyr208Leu + Leu211Met + Gly213Gln
	Pro195Ser + Val197Pro + Pro204Glu + Leu211Cys
20	Pro195Gly + Gly196Ser + Val199Gln + Thr214Asp
	Val197Asn + Gly205Asn + Thr207Ser + Ser210Asp
	Ala194His + Tyr203His + Gly205Pro + Ser206Glu
	Pro195Asn + Asn198Gln + Val199Gln + Leu211Gln
	Val197Pro + Thr202Gly + Pro204Gly + Thr207Ser
25	Thr202Pro + Tyr203Leu + Leu211Ala + Thr214Asp
	Pro195Asn + Val199His + Tyr203Met + Thr207Glu
	Val199Cys + Pro204Gly + Thr207Ser + Thr214Asp
	Ala194Ser + Pro195Gln + Tyr203Thr + Tyr208Pro
	Val199Cys + Tyr203Cys + Ser206Asp + Tyr208Ala
30	Val197Thr + Thr202Gln + Leu211Glu + Asn212Ser
	Gly196Ser + Val199Pro + Ala209Gln + Leu211Asn
	Ala194Gly + Ala209Gln + Asn212Asp + Thr214Gln
	Ala194Gln + Thr207Asn + Ala209Pro + Leu211His
	Gly196Ser + Val197Ser + Thr202Gln + Leu211Gln
35	Ala194Thr + Val197Ser + Thr202Pro + Thr207Pro
	Gly196Pro + Thr202Pro + Gly205Glu + Gly213Pro
	Ala194Pro + Pro195Gly + Val197Asn + Val199Cys
	Thr202Gly + Thr207Ser + Tyr208Pro + Leu211Pro
	Thr202Asn + Pro204Asp + Thr207Ser + Gly213Asn
40	Gly196Gln + Val197Cys + Tyr203Met + Ala209Asn
	Gly196Gln + Ser206Glu + Thr207Ser + Gly213Pro
	Pro195Ser + Val199Cys + Ser210Glu + Asn212Ser
	Ala194Ser + Ser206Asp + Asn212Ser + Thr214Gln
	Gly196Gln + Gln200Ser + Thr202Gln + Ala209Ser
45	Gly196Gln + Tyr203Thr + Thr207Gln + Asn212Glu
	Ala194Pro + Thr202Gln + Tyr208Cys + Leu211Pro
	Gln200Asp + Tyr203Cys + Leu211Gln + Asn212Gln
	Gly196Asn + Tyr203Met + Leu211Asn + Gly213Asn
	Pro195Gln + Gly196Asn + Gln200Ser + Thr202Gln
50	Thr202Pro + Tyr203Gly + Asn212Ser + Thr214Gly
	Gln200Asp + Tyr203Thr + Pro204Gln + Gly205Ser
	Gly196Ser + Thr202Pro + Pro204Asn + Ala209Ser
	Gly196Ser + Gln200Ser + Pro204Gly + Ser210Asp
	Ala194Gln + Gln200Asn + Thr202Ser + Gly205Gln

5      Pro195Gly + Gln200Asn + Thr207Asp + Ala209His  
Gln200Asn + Thr202Ser + Tyr203Ala + Thr214Gly  
Gln200Glu + Ala209Pro + Leu211Ile + Gly213Asn  
Ala194Asn + Thr202Ser + Tyr203Met + Ser206Glu  
Val197Asn + Asn198Ser + Thr207Gln + Thr214Glu  
Val197Met + Gln200Ser + Pro204Glu + Ala209Pro  
Gly196Asn + Val199Asn + Leu211Glu + Asn212Gln  
Pro195Gln + Thr202Gln + Gly205Asp + Tyr208Asn  
Thr202Pro + Ser206Asp + Leu211Ile + Gly213Ser  
10      Gly196Ser + Gln200Glu + Thr202Gln + Tyr208Met  
Asn198Glu + Tyr203Thr + Tyr208Asn + Gly213Gln  
Asn198Ser + Ser210Glu + Leu211His + Thr214Pro  
Gly196Ser + Val197Gly + Pro204Glu + Thr214Ser  
Tyr203Met + Ser206Glu + Tyr208Ser + Ala209His  
15      Gly196Gln + Gln200Ser + Leu211Cys + Gly213Pro  
Ala194Gly + Val199Asn + Thr202Asn + Gly213Pro  
Asn198Ser + Thr202Asn + Tyr203Gly + Ser210Glu  
Val199Gln + Gln200Ser + Tyr203His + Thr214Asp  
Thr202Gln + Ser206Asp + Thr207Ser + Thr214Gly  
20      Ala194Thr + Tyr203Gln + Thr207Asp + Leu211Ile  
Ala209Ser + Ser210Asp + Asn212Gln + Gly213Ser  
Val197Ser + Tyr203Gly + Ser206Glu + Asn212Gln  
Ala194Ser + Asn198Ser + Gly205Gln + Ser210Asp  
Ala194Gln + Pro195Ser + Val197Gln + Pro204Asn  
25      Ala194Gly + Asn198Ser + Ala209Glu + Thr214Asn  
Val199Met + Ser206Asp + Gly213Asn + Thr214Ser  
Thr202Gln + Gly205Ser + Leu211Asp + Asn212Glu  
Pro195Ser + Ala209Gly + Gly213Asp + Thr214Asp  
Thr202Gln + Thr207Pro + Gly213Glu + Thr214Asp  
30      Ala194Ser + Thr207Pro + Ala209Glu + Ser210Glu  
Gln200Asn + Thr202Gln + Ala209Glu + Ser210Glu  
Gln200Ser + Ala209Asp + Ser210Asp + Asn212Gln  
Gly205Asn + Ala209Asp + Ser210Glu + Asn212Gln  
Pro204Gly + Leu211Gln + Asn212Glu + Gly213Asp  
35      Tyr203Gly + Pro204Asp + Gly205Glu + Leu211Ala  
Thr202Ser + Pro204Glu + Gly205Asp + Thr207Gln  
Thr202Gly + Pro204Asp + Gly205Glu + Gly213Asn  
Val199Gly + Ser210Asp + Leu211Asp + Asn212Gln  
Gly196Pro + Thr202Ser + Gly205Asp + Ser206Glu  
40      Val197Thr + Gly205Asp + Ser206Glu + Gly213Gln  
Pro204Asn + Gly205Glu + Ser206Glu + Asn212Ser  
Ala194His + Gly205Asp + Ser206Asp + Leu211His  
Pro195Asn + Ser206Glu + Thr207Asp + Asn212Ser  
Tyr203Met + Ser206Glu + Thr207Glu + Leu211Gly  
45      Gly196Gln + Pro204Asn + Ser206Asp + Thr207Glu  
Ala194Thr + Val197Cys + Ser206Asp + Thr207Asp  
Ala194Pro + Tyr208Asp + Ala209Asp + Gly213Gln  
Gln200Asp + Tyr208Thr + Ala209Ser + Ser210Glu  
Val199Asn + Gln200Asp + Thr202Gln + Ser210Asp  
50      Ala194Ser + Gln200Glu + Thr202Gly + Ser210Glu  
Val199Cys + Gln200Asp + Ala209Gln + Ser210Asp  
Ala194Gln + Val197Asp + Tyr208Gln + Asn212Asp  
Pro195Gly + Asn198Glu + Ser210Asp + Leu211Gln  
Ala194Pro + Pro195Asn + Asn198Asp + Ser210Asp

5 Pro195Gln + Asn198Glu + Val199Ser + Ser210Asp  
 Val197His + Asn198Glu + Thr207Pro + Ser210Asp  
 Gly196Pro + Val197Gly + Asn198Asp + Ser210Glu  
 Asn198Glu + Ala209Gln + Leu211Glu + Thr214Pro  
 Gln200Glu + Tyr203Asn + Thr207Gln + Ala209Glu  
 Pro195Ser + Gly196Ser + Gln200Glu + Ala209Asp  
 Pro195Ser + Gln200Asp + Ala209Glu + Leu211Ala

TABLE 27

Loop 6 - Quintuple Mutation Variants	
10	Val197Ala + Asn198Gln + Thr207Ser + Tyr208Ile + Asn212Asp
	Ala194Gly + Gly196Ser + Val197Gly + Gln200Glu + Leu211Gly
	Gln200Asp + Thr202Ser + Tyr203Ser + Pro204Ser + Asn212Ser
	Pro195Ser + Val199Ala + Gly205Asn + Ser206Glu + Gly213Ser
15	Val199His + Pro204Asp + Gly205Pro + Leu211Gly + Thr214Ser
	Ala194Gly + Thr207Pro + Ser210Glu + Leu211Cys + Gly213Gln
	Pro195Asn + Gly196Pro + Asn198Ser + Thr202Gly + Gly213Asn
	Gly196Ser + Val197Gln + Pro204Ser + Gly205Asp + Leu211Gly
	Pro195Ser + Gly196Gln + Pro204Asn + Gly213Asp + Thr214Gly
20	Pro195Gly + Val199Asn + Gln200Asn + Ser210Glu + Leu211Gly
	Val197Ser + Val199Gly + Tyr203Thr + Gly205Ser + Asn212Glu
	Pro195Asn + Asn198Asp + Thr202Ser + Ala209His + Asn212Gln
	Gly196Asn + Gly205Pro + Thr207Pro + Asn212Asp + Gly213Gln
	Val197Gly + Asn198Asp + Val199His + Gly205Gln + Tyr208His
25	Tyr203Val + Thr207Gly + Ala209Ser + Asn212Glu + Gly213Gln
	Ala194Thr + Pro195Gly + Gly196Ser + Tyr203Asn + Asn212Ser
	Pro195Gln + Thr202Gly + Tyr203Leu + Pro204Gly + Ser210Asp
	Pro195Asn + Val199Ala + Thr202Asn + Tyr208Pro + Ser210Asp
	Ala194Pro + Pro195Gly + Asn198Glu + Pro204Asn + Tyr208Pro
30	Ala194Ser + Val197Thr + Asn198Ser + Thr202Pro + Asn212Ser
	Gly196Ser + Val199Pro + Thr207Ser + Tyr208Thr + Asn212Gln
	Gly205Glu + Tyr208Gly + Leu211Ile + Asn212Ser + Thr214Gly
	Gln200Ser + Thr202Gly + Thr207Asp + Leu211Ser + Thr214Gln
	Pro195Asn + Thr207Asp + Tyr208Asn + Asn212Ser + Gly213Asn
35	Pro195Asn + Tyr203Ser + Pro204Glu + Leu211Val + Asn212Gln
	Gln200Asp + Gly205Ser + Tyr208Met + Leu211Asn + Asn212Ser
	Ala194Ser + Val199Pro + Gly205Glu + Tyr208Met + Gly213Ser
	Asn198Ser + Gln200Glu + Thr202Gly + Ala209Gln + Leu211Asn
	Gly196Pro + Asn198Glu + Thr202Ser + Ala209Thr + Thr214Gly
40	Ala194Gln + Pro195Gly + Gly196Asn + Tyr203Ile + Thr214Pro
	Pro195Gln + Val197Cys + Thr202Gln + Tyr203Cys + Tyr208His
	Val199Pro + Pro204Gly + Gly205Ser + Thr207Ser + Gly213Asp
	Tyr203Gly + Pro204Ser + Gly205Asn + Ala209Thr + Ser210Asp
	Ala194Ser + Thr207Ser + Tyr208Cys + Leu211Pro + Thr214Pro
45	Pro195Gly + Val199His + Gly205Glu + Ala209Asn + Leu211Pro
	Pro204Asn + Tyr208Ile + Ala209Gly + Leu211Gln + Gly213Glu
	Ala194Gln + Val197Cys + Asn198Ser + Pro204Gly + Asn212Glu
	Asn198Glu + Pro204Asn + Ala209Thr + Leu211Ser + Gly213Pro
	Gly196Ser + Val199Ser + Pro204Asp + Thr207Gly + Tyr208Ile
50	Gln200Glu + Thr202Ser + Tyr203Ala + Pro204Gly + Tyr208Met
	Asn198Gln + Thr202Pro + Gly205Asn + Thr207Glu + Thr214Pro
	Gly196Ser + Val199Pro + Ala209Gln + Ser210Asp + Leu211Asn

Ala194Gly + Thr207Asn + Ala209Gln + Asn212Asp + Thr214Gln  
 Pro195Gly + Asn198Gln + Val199Thr + Tyr208His + Leu211Ile  
 Ala194Pro + Thr202Gly + Thr207Ser + Tyr208Pro + Leu211Pro  
 Gly196Ser + Val197Ala + Gln200Asn + Thr202Pro + Ser210Glu  
 5 Val197Gln + Thr202Asn + Tyr203Asn + Gly205Asp + Tyr208Ala  
 Gly196Pro + Tyr203Ile + Pro204Asn + Leu211Ala + Thr214Gln  
 Ala194Asn + Gln200Asn + Pro204Gln + Ala209Thr + Gly213Ser  
 Ala194His + Pro195Gly + Gln200Ser + Leu211Asn + Asn212Ser  
 Ala194Ser + Gly196Ser + Gln200Asn + Ser206Asp + Thr207Gly  
 10 Gly196Asn + Tyr203Asn + Tyr208Ala + Ala209Pro + Gly213Gln  
 Ala194Thr + Val199Asn + Tyr203Ser + Tyr208His + Asn212Asp  
 Val197Glu + Val199Ala + Tyr208Gln + Ala209Gly + Leu211Val  
 Gln200Asn + Thr202Ser + Tyr203Ala + Thr207Gln + Thr214Gly  
 Val197Asn + Asn198Ser + Thr202Pro + Thr207Gln + Thr214Glu  
 15 Pro195Gln + Asn198Glu + Gln200Ser + Tyr203Cys + Gly213Gln  
 Gly196Gln + Asn198Ser + Thr202Asn + Tyr203Ser + Gly205Pro  
 Ala194Thr + Thr207Pro + Tyr208Ile + Ala209Pro + Thr214Asn  
 Asn198Ser + Val199Pro + Thr202Asn + Tyr203Gly + Asn212Glu  
 Ala194Asn + Thr202Asn + Ser210Asp + Leu211Val + Asn212Ser  
 20 Val197Ser + Val199His + Thr202Asn + Ser210Asp + Leu211Cys  
 Gly196Pro + Val197Cys + Val199Met + Tyr203Ser + Leu211Asn  
 Ala194Gly + Gly196Pro + Val199His + Thr202Pro + Ser210Glu  
 Gly196Ser + Val197His + Asn198Glu + Thr202Pro + Asn212Ser  
 Ala194His + Gly196Asn + Val197Gln + Thr202Ser + Gly205Glu  
 25 Pro195Gly + Gly196Pro + Asn198Ser + Gln200Ser + Thr202Pro  
 Val197Asn + Asn198Asp + Gln200Asn + Thr202Gln + Tyr208Ala  
 Pro195Asn + Thr202Ser + Pro204Asp + Tyr208Ala + Ala209Ser  
 Tyr203His + Pro204Asn + Gly205Asp + Thr207Gln + Leu211Gly  
 Ala194Asn + Pro195Gln + Gly196Gln + Pro204Asn + Gly213Glu  
 30 Val197Thr + Val199His + Ser206Glu + Gly213Asn + Thr214Asn  
 Gly196Gln + Val197Asn + Tyr203Thr + Pro204Ser + Thr214Asp  
 Gly196Asn + Val199Gly + Gln200Ser + Tyr203Asn + Gly213Glu  
 Ala194Thr + Gln200Asn + Thr202Ser + Gly205Gln + Gly213Asp  
 Pro195Ser + Asn198Ser + Val199Gly + Tyr203Val + Thr207Glu  
 35 Ala194Asn + Pro195Gly + Thr202Gly + Tyr203Gly + Leu211Asp  
 Pro195Ser + Val197Ser + Tyr203Cys + Gly205Glu + Ala209Thr  
 Gly196Gln + Val197Gly + Gly205Ser + Tyr208Leu + Thr214Asp  
 Ala194Gln + Gly196Asn + Tyr203Pro + Pro204Ser + Gly213Asp  
 Gly196Gln + Thr202Gly + Tyr203Cys + Pro204Gln + Ser206Glu  
 40 Val197Pro + Val199Met + Tyr203Val + Pro204Gly + Thr214Asp  
 Gly196Pro + Gln200Asn + Tyr203Cys + Ala209Thr + Thr214Asn  
 Asn198Gln + Pro204Asn + Thr207Gln + Gly213Pro + Thr214Asp  
 Pro195Gly + Val197Ser + Val199Ala + Thr207Asp + Leu211Gly  
 Pro195Gln + Asn198Gln + Ser206Asp + Thr207Ser + Ala209Ser  
 45 Ala194Ser + Tyr203Leu + Ala209Asn + Ser210Asp + Thr214Ser  
 Val199His + Thr202Ser + Gly205Asn + Leu211Pro + Asn212Gln  
 Gly196Ser + Asn198Ser + Thr207Asn + Tyr208Gln + Ser210Glu  
 Pro195Asn + Asn198Gln + Thr207Ser + Gly213Asn + Thr214Gly  
 Gly196Gln + Gln200Ser + Pro204Asn + Gly205Gln + Gly213Asp  
 50 Pro204Asn + Ser206Glu + Tyr208Leu + Ala209Gly + Asn212Gln  
 Val197Thr + Val199Gly + Gln200Asp + Thr202Pro + Thr207Gly  
 Pro195Gln + Val199Ser + Thr202Asn + Gly205Asp + Ala209Gln  
 Gly196Gln + Tyr203Thr + Tyr208Cys + Leu211Ala + Thr214Glu  
 Asn198Gln + Gln200Ser + Pro204Gln + Asn212Ser + Gly213Pro



5      Val197Pro + Val199Asn + Thr202Gln + Tyr203Asn + Gly213Ser  
        Ala194Ser + Gln200Ser + Thr202Gln + Thr207Gln + Ala209Glu  
        Thr202Gly + Tyr203Thr + Tyr208Pro + Gly213Asp + Thr214Glu  
        Val197Gln + Pro204Gly + Gly205Ser + Ala209Glu + Ser210Asp  
        Ala194Ser + Val199Ala + Tyr208Ala + Ala209Glu + Ser210Glu  
        Val199Ala + Gln200Ser + Gly205Pro + Asn212Glu + Gly213Asp  
        Ala194Pro + Thr202Ser + Pro204Glu + Gly205Asp + Ala209Pro  
        Pro204Glu + Gly205Glu + Thr207Gly + Tyr208Ser + Leu211Ile  
        Pro195Gln + Val197Thr + Pro204Glu + Gly205Asp + Leu211Val  
 10     Val199His + Gly205Asp + Ser206Glu + Ala209Gln + Asn212Gln  
        Tyr203Ala + Gly205Glu + Ser206Asp + Tyr208Ile + Asn212Gln  
        Pro195Asn + Gly205Glu + Ser206Asp + Tyr208Ser + Ala209Ser  
        Val197His + Pro204Gly + Gly205Glu + Ser206Asp + Tyr208Cys  
        Thr202Asn + Pro204Ser + Gly205Asp + Ser206Asp + Tyr208Thr  
 15     Ala194Asn + Thr202Ser + Tyr203Gly + Ser206Glu + Thr207Glu  
        Pro195Ser + Gly196Gln + Val199Thr + Ser206Glu + Thr207Asp  
        Gly196Pro + Ser206Asp + Thr207Glu + Tyr208Thr + Leu211His  
        Ala194His + Gly196Gln + Pro204Asn + Tyr208Asp + Ala209Asp  
        Val199Ala + Tyr203Pro + Tyr208Asp + Ala209Asp + Asn212Gln  
 20     Val199Ala + Thr202Ser + Thr207Glu + Tyr208Asp + Thr214Ser  
        Gly196Asn + Thr202Gln + Pro204Ser + Thr207Asp + Tyr208Asp  
        Pro195Gln + Gln200Glu + Thr202Pro + Tyr203Pro + Ser210Asp  
        Gly196Pro + Val197Thr + Asn198Gln + Gln200Asp + Ser210Asp  
        Gly196Gln + Asn198Glu + Thr207Pro + Tyr208Ser + Ser210Glu  
 25     Ala194Pro + Asn198Glu + Thr207Pro + Ala209His + Ser210Glu  
        Asn198Gly + Asn198Glu + Thr207Gly + Tyr208Cys + Ser210Asp  
        Asn198Asp + Val199Thr + Leu211Asp + Asn212Ser + Thr214Gly  
        Gly196Pro + Asn198Gln + Gln200Glu + Tyr203Asn + Ala209Glu  
 30     Gly196Pro + Tyr203Thr + Pro204Asp + Ser206Asp + Ala209Gln  
        Pro195Asn + Gly196Pro + Asn198Asp + Ala209Pro + Asn212Glu  
        Pro195Asn + Gly196Gln + Gly205Asp + Thr207Glu + Leu211Thr  
        Thr202Asn + Pro204Asp + Thr207Asp + Tyr208Met + Thr214Pro  
        Tyr203Gly + Pro204Asp + Thr207Asp + Asn212Gln + Thr214Gly  
 35     Pro195Ser + Tyr203His + Pro204Glu + Thr207Asp + Gly213Gln  
        Asn198Glu + Gln200Glu + Tyr203Cys + Pro204Gly + Asn212Gln  
        Val197Glu + Val199Gly + Thr202Gly + Tyr203Ser + Gly213Glu  
        Pro204Gly + Thr207Asn + Ala209His + Ser210Asp + Asn212Asp  
        Pro195Gly + Gly196Gln + Thr202Gln + Ser210Asp + Asn212Asp  
 40     Thr202Asn + Tyr203Asn + Gly205Pro + Ser210Asp + Asn212Asp  
        Thr202Gln + Tyr203Gln + Ser210Asp + Asn212Asp + Gly213Gln  
        Gly196Ser + Ser210Asp + Leu211Gly + Asn212Asp + Thr214Gln  
        Gly196Pro + Ala209Ser + Ser210Glu + Leu211Asn + Asn212Asp  
        Gly205Ser + Ala209Pro + Ser210Glu + Asn212Asp + Thr214Pro  
 45     Pro195Gly + Tyr203Gly + Ser210Asp + Asn212Asp + Thr214Ser  
        Pro195Gln + Val197Asn + Val199Cys + Tyr208Glu + Ser210Asp  
        Val197Ser + Tyr203Gly + Pro204Glu + Thr207Ser + Tyr208Asp  
        Pro195Ser + Gly196Asn + Val197Asp + Val199Gly + Ser210Glu  
        Gly196Pro + Val197Asp + Thr202Pro + Ser210Glu + Thr214Pro  
 50     Val197Glu + Val199His + Tyr208Thr + Leu211Gln + Thr214Glu  
        Asn198Asp + Val199Asn + Thr207Asn + Ala209His + Gly213Asp  
        Val197His + Asn198Glu + Tyr203Gln + Tyr208Cys + Gly213Asp  
        Pro195Asn + Val197Asn + Asn198Glu + Thr202Pro + Gly213Asp  
        Pro195Ser + Asn198Glu + Tyr203Ser + Gly205Ser + Ala209Asp

	Pro195Gly + Gly196Asn + Asn198Asp + Val199Met + Ala209Glu
	Pro195Gln + Asn198Glu + Thr202Asn + Pro204Gly + Ala209Asp
	Val197Glu + Gln200Glu + Thr202Gly + Tyr208Ala + Gly213Gln
	Val197Asp + Gln200Asp + Thr207Ser + Tyr208Ile + Ala209Ser
5	Val197Asp + Gln200Glu + Ala209Ser + Leu211Gly + Asn212Ser
	Val197Glu + Gln200Asp + Thr202Asn + Gly205Gln + Asn212Gln
	Ala194Pro + Gly196Pro + Val197Met + Gln200Glu + Asn212Asp
	Gly196Gln + Gln200Glu + Thr202Pro + Tyr203Ile + Asn212Glu
	Ala194His + Gln200Asp + Asn212Glu + Gly213Pro + Thr214Asn
10	Val199Pro + Gln200Glu + Pro204Gly + Asn212Glu + Gly213Ser
	Asn198Ser + Gln200Asp + Gly205Pro + Thr207Ser + Asn212Glu
	Ala194Asn + Gln200Asp + Gly205Asn + Tyr208Asn + Asn212Asp
	Pro195Asn + Gln200Ser + Thr202Pro + Pro204Asp + Ala209Glu
	Pro195Ser + Thr202Asn + Tyr203Cys + Pro204Glu + Ala209Asp
15	Ala194His + Asn198Ser + Thr202Asn + Pro204Glu + Ala209Asp
	Ala194His + Pro204Asn + Ser206Glu + Ala209Glu + Thr214Ser
	Pro195Gly + Val197Cys + Ser206Glu + Ala209Glu + Asn212Ser
	Thr202Gly + Ser206Glu + Thr207Ser + Tyr208Met + Ala209Glu
	Ala194Gly + Gln200Glu + Thr207Asp + Leu211Ser + Thr214Pro
20	Pro195Asn + Val199Ser + Gln200Glu + Gly205Asn + Thr207Asp
	Ala194Gln + Asn198Gln + Gly205Pro + Ser210Asp + Gly213Asp
	Gly196Ser + Thr207Gly + Ser210Glu + Leu211Thr + Gly213Asp
	Asn198Gln + Pro204Gln + Thr207Asp + Tyr208Thr + Ser210Glu
	Ala194Ser + Gly196Gln + Thr207Glu + Ser210Glu + Leu211Thr
25	Thr207Glu + Ser210Glu + Asn212Ser + Gly213Ser + Thr214Ser
	Val197Cys + Thr207Glu + Ala209His + Ser210Glu + Asn212Ser
	Asn198Glu + Val199Met + Tyr208Ala + Gly213Gln + Thr214Asp
	Ala194Gln + Asn198Glu + Ala209Pro + Leu211His + Thr214Asp
	Asn198Asp + Thr207Pro + Leu211Gly + Asn212Ser + Thr214Asp
30	Ala194Ser + Gly196Asn + Asn198Asp + Pro204Gln + Thr214Glu

TABLE 28

Loop 6 - Sextuple Mutation Variants	
35	Ala194Gln + Val197Ser + Thr202Ser + Tyr203Ser + Ala209Ser + Asn212Asp Pro195Ser + Val199Cys + Gly205Ser + Leu211Thr + Asn212Glu + Gly213Pro Val197Thr + Asn198Ser + Val199Pro + Gln200Ser + Thr202Ser + Leu211Asn
40	Thr202Gly + Pro204Ser + Gly205Asn + Tyr208Asp + Gly213Gln + Thr214Pro Val197Gly + Gln200Glu + Thr202Gly + Tyr208Ala + Leu211Val + Asn212Ser Ala194Pro + Gly196Ser + Val199Cys + Tyr203Ala + Pro204Ser + Ala209Asp
45	Pro195Asn + Val199Pro + Thr202Asn + Tyr203Gly + Thr207Asp + Gly213Asn Ala194Gln + Thr202Ser + Tyr203Ile + Pro204Asn + Gly205Asp + Tyr208Ser
50	Ala194Gln + Val197His + Ala209Ser + Ser210Asp + Leu211Gly + Asn212Gln Thr202Gly + Gly205Pro + Ser210Asp + Leu211Ala + Gly213Ser

- + Thr214Gln  
Ala194His + Gln200Ser + Thr202Gly + Pro204Ser + Tyr208His  
+ Gly213Glu  
Ala194Asn + Pro195Gln + Gly205Asn + Tyr208Ser + Leu211Val  
+ Thr214Pro  
5 Gly196Pro + Asn198Ser + Gln200Ser + Thr202Asn + Tyr208Ile  
+ Thr214Glu  
Pro195Asn + Gly205Pro + Thr207Gln + Ser210Glu + Leu211Asn  
+ Gly213Pro  
10 Ala194Ser + Val197Gly + Asn198Asp + Tyr203Cys + Gly205Gln  
+ Leu211Met  
Ala194Ser + Val197Asp + Val199Gln + Tyr203Cys + Gly205Ser  
+ Gly213Gln  
Ala194Pro + Thr202Gly + Thr207Ser + Tyr208Met + Leu211Pro  
+ Gly213Glu  
15 Ala194Thr + Pro195Asn + Gly196Gln + Gln200Ser + Thr202Asn  
+ Pro204Glu  
Ala194Gln + Gly196Asn + Val197Ser + Asn198Ser + Val199Thr  
+ Ser210Glu  
20 Gly196Ser + Val199Gly + Tyr203Ile + Pro204Asn + Leu211Asp  
+ Thr214Gln  
Ala194Asn + Gln200Asn + Pro204Gln + Ala209Thr + Leu211Asn  
+ Gly213Ser  
Ala194His + Pro195Gly + Gly196Asn + Gln200Ser + Tyr203Ser  
+ Asn212Ser  
25 Ala194Pro + Val197Met + Val199Asn + Thr202Ser + Ser206Asp  
+ Thr207Asn  
Pro195Gln + Thr202Pro + Gly205Asn + Ala209Pro + Asn212Ser  
+ Thr214Asn  
30 Pro195Asn + Asn198Glu + Val199His + Pro204Asn + Thr207Gly  
+ Leu211Met  
Gly196Ser + Pro204Asn + Thr207Pro + Asn212Asp + Gly213Ser  
+ Thr214Pro  
Ala194Ser + Pro195Ser + Val197Cys + Pro204Asn + Thr207Glu  
+ Gly213Gln  
35 Ala194His + Pro195Gly + Val197Gly + Val199Gln + Thr207Gly  
+ Asn212Glu  
Ala194Gln + Pro195Ser + Val197Ser + Tyr203Met + Leu211Glu  
+ Gly213Asn  
40 Ala194Asn + Gly196Asn + Pro204Asp + Thr207Pro + Tyr208Ser  
+ Thr214Ser  
Pro195Asn + Thr202Gly + Gly205Asp + Thr207Gln + Tyr208Thr  
+ Ala209Asn  
Ala194His + Pro195Gly + Val197His + Gly205Gln + Ala209Glu  
+ Thr214Asn  
45 Ala194Gln + Asn198Gln + Tyr203Met + Ala209Gln + Ser210Glu  
+ Leu211Gly  
Ala194Pro + Gly196Ser + Gln200Ser + Thr202Pro + Thr207Asp  
+ Ala209Gly  
50 Pro195Gln + Gly196Ser + Asn198Gln + Gly205Ser + Ser210Asp  
+ Leu211Val  
Asn198Gln + Val199Met + Gln200Glu + Thr202Gly + Gly205Asn  
+ Tyr208Ala  
Ala194Pro + Val199Ala + Pro204Asn + Thr207Gln + Leu211Glu

+ Gly213Ser  
 Gly196Pro + Val197Thr + Val199His + Gly205Asn + Ser206Asp  
 + Thr207Asn  
 Pro195Asn + Gly196Ser + Thr202Asn + Tyr203Gly + Leu211Asn  
 5 + Asn212Glu  
 Ala194Ser + Pro195Gln + Val197Cys + Ser206Glu + Tyr208His  
 + Asn212Gln  
 Pro195Gln + Gly196Pro + Val197Thr + Ser210Asp + Gly213Ser  
 + Thr214Pro  
 10 Ala194Gly + Pro195Ser + Thr202Gly + Pro204Glu + Tyr208Thr  
 + Gly213Pro  
 Ala194Asn + Pro195Asn + Gly196Asn + Gly205Pro + Ala209Asn  
 + Ser210Asp  
 Pro195Ser + Val199Gln + Tyr208Asn + Ala209Gln + Leu211Gly  
 15 + Gly213Gln  
 Asn198Asp + Val199Ala + Thr202Asn + Ala209Pro + Asn212Gln  
 + Thr214Gly  
 Pro195Asn + Gly196Gln + Val199Pro + Ser206Glu + Tyr208Ala  
 + Leu211Val  
 20 Ala194Gln + Pro195Ser + Asn198Asp + Val199Ser + Thr202Pro  
 + Ala209Gln  
 Pro195Gly + Val199Met + Gly205Glu + Thr207Asn + Ala209Pro  
 + Gly213Pro  
 Pro195Asn + Gly196Gln + Gln200Asp + Thr207Asn + Ala209Gly  
 25 + Asn212Ser  
 Gly196Gln + Val197His + Thr207Asn + Ser210Asp + Leu211Ala  
 + Gly213Gln  
 Ala194Gly + Val197Thr + Tyr203Met + Gly205Glu + Ala209Gly  
 + Thr214Gly  
 30 Gln200Glu + Thr202Asn + Tyr203Gly + Thr207Asn + Asn212Gln  
 + Gly213Pro  
 Gly196Asn + Thr202Ser + Pro204Glu + Leu211Pro + Gly213Ser  
 + Thr214Asn  
 Pro195Gly + Val197Asn + Val199Gln + Gln200Asn + Thr207Gly  
 35 + Asn212Ser  
 Pro195Gln + Gly196Pro + Val197His + Tyr203Gly + Ser206Asp  
 + Thr214Ser  
 Gly196Gln + Tyr203His + Thr207Asn + Leu211Asn + Gly213Pro  
 + Thr214Asp  
 40 Ala194Asn + Asn198Ser + Gln200Asn + Thr202Ser + Tyr203Ile  
 + Ala209Thr  
 Ala194His + Val197Cys + Gln200Ser + Tyr203Gly + Thr207Gly  
 + Ala209His  
 Asn198Gln + Val199Thr + Gln200Asp + Pro204Asn + Asn212Ser  
 45 + Gly213Gln  
 Gly196Pro + Tyr203Gln + Pro204Ser + Thr207Asn + Tyr208Met  
 + Leu211Asp  
 Gly196Ser + Val197Met + Asn198Ser + Thr202Asn + Thr207Asp  
 + Asn212Gln  
 50 Pro195Gly + Gly196Pro + Thr202Asn + Tyr203Ile + Gly213Asp  
 + Thr214Asn  
 Pro195Gly + Gly196Pro + Gln200Glu + Pro204Ser + Tyr208Ile  
 + Gly213Ser  
 Val199Ala + Thr202Gln + Gly205Ser + Ala209Asn + Ser210Glu

+ Leu211Cys  
Ala194His + Pro195Ser + Val199Thr + Gln200Glu + Tyr203Pro  
+ Tyr208Cys  
Gly196Gln + Pro204Gly + Ser206Asp + Thr207Pro + Ala209Gln  
5 + Gly213Ser  
Pro195Asn + Thr202Asn + Pro204Asn + Ala209Gln + Asn212Asp  
+ Gly213Gln  
Gly196Ser + Asn198Gln + Tyr203His + Asn212Ser + Gly213Asp  
+ Thr214Gly  
10 Ala194Gln + Val197Pro + Val199Gln + Gln200Asn + Tyr208Glu  
+ Thr214Ser  
Val199Cys + Gln200Asn + Pro204Gly + Ser206Asp + Thr207Ser  
+ Asn212Ser  
Ala194Gln + Gly196Gln + Val199His + Thr202Pro + Tyr203Thr  
15 + Gly205Ser  
Asn198Gln + Val199Gly + Thr202Ser + Ser210Asp + Asn212Ser  
+ Gly213Gln  
Val199Thr + Gln200Asn + Pro204Ser + Tyr208Val + Asn212Gln  
+ Thr214Asp  
20 Ala194His + Gly196Gln + Val199Gln + Thr202Gly + Pro204Asp  
+ Leu211Cys  
Ala194Ser + Val197Gly + Pro204Gln + Ala209Pro + Ser210Glu  
+ Thr214Gly  
Ala194Gln + Gly196Pro + Tyr203Gln + Thr207Pro + Tyr208Pro  
25 + Ala209Asn  
Gly196Ser + Thr202Asn + Tyr208Ile + Ala209Gln + Leu211Ala  
+ Gly213Glu  
Pro195Asn + Gly196Pro + Val197Glu + Gln200Asn + Tyr203Ser  
+ Tyr208Ser  
30 Gly196Ser + Val199His + Gly205Asp + Tyr208Val + Ala209Gly  
+ Thr214Pro  
Val197Ser + Val199Met + Gln200Asn + Thr202Ser + Tyr208Cys  
+ Gly213Gln  
Ala194Ser + Gly196Gln + Val199Pro + Leu211Val + Gly213Glu  
35 + Thr214Gly  
Ala194Gln + Asn198Gln + Val199Cys + Thr207Gly + Leu211Asp  
+ Asn212Glu  
Ala194Thr + Asn198Gln + Thr202Asn + Pro204Ser + Gly213Glu  
+ Thr214Asp  
40 Ala194Thr + Pro195Ser + Val199Ala + Ala209Glu + Ser210Glu  
+ Leu211Cys  
Tyr203Leu + Gly205Ser + Ala209Glu + Ser210Glu + Asn212Ser  
+ Gly213Asn  
Val197Glu + Asn198Glu + Tyr203Pro + Thr207Gly + Ala209Ser  
45 + Asn212Ser  
Val197Asp + Asn198Asp + Val199His + Thr202Ser + Pro204Gln  
+ Ala209Asn  
Gly196Asn + Val197Glu + Asn198Asp + Gln200Ser + Pro204Asn  
+ Thr207Gly  
50 Pro195Gln + Thr202Ser + Tyr203Ser + Thr207Gln + Asn212Asp  
+ Gly213Glu  
Gly196Gln + Tyr203His + Gly205Ser + Leu211Asn + Asn212Asp  
+ Gly213Glu  
Asn198Ser + Gly205Ser + Tyr208Ile + Asn212Glu + Gly213Glu

+ Thr214Pro  
 Ala194Ser + Gly196Gln + Pro204Glu + Gly205Asp + Tyr208Asn  
 + Thr214Asn  
 Ala194Ser + Pro195Gly + Val199Cys + Pro204Asp + Gly205Glu  
 5 + Asn212Gln  
 Ala194Thr + Val199Pro + Gly205Asn + Ala209Gln + Ser210Glu  
 + Leu211Asp  
 Thr202Gln + Thr207Gly + Ser210Asp + Leu211Glu + Gly213Gln  
 + Thr214Ser  
 10 Thr202Gln + Gly205Asp + Ser206Glu + Tyr208Met + Gly213Gln  
 + Thr214Asn  
 Ala194Gly + Gly205Glu + Ser206Glu + Ala209Gly + Leu211Gln  
 + Thr214Gly  
 Gln200Ser + Thr202Ser + Tyr203Gly + Gly205Asp + Ser206Glu  
 15 + Thr214Gln  
 Val197Asn + Thr202Ser + Gly205Glu + Ser206Glu + Ala209His  
 + Thr214Ser  
 Ala194Gln + Thr202Ser + Tyr203Gly + Ser206Asp + Thr207Glu  
 + Gly213Ser  
 20 Gly196Pro + Val199Gln + Tyr203Met + Ser206Glu + Thr207Asp  
 + Ala209Thr  
 Pro195Gly + Gly196Pro + Thr202Ser + Pro204Gln + Tyr208Asp  
 + Ala209Glu  
 Pro195Gln + Gly196Pro + Val197Pro + Gln200Asp + Gly205Ser  
 25 + Ser210Asp  
 Gly196Ser + Asn198Glu + Gly205Pro + Ala209His + Ser210Glu  
 + Asn212Gln  
 Ala194Thr + Asn198Asp + Thr202Ser + Tyr203Cys + Ala209Pro  
 + Ser210Glu  
 30 Gly196Pro + Asn198Glu + Gly205Ser + Ser210Glu + Leu211Asn  
 + Thr214Asn  
 Ala194Pro + Gly196Ser + Asn198Glu + Gly205Ser + Thr207Pro  
 + Ser210Glu  
 Gly196Ser + Val197His + Asn198Asp + Gln200Ser + Thr207Gln  
 + Ser210Asp  
 35 Asn198Glu + Val199Met + Gly205Pro + Ala209His + Ser210Glu  
 + Thr214Pro  
 Val197Ser + Asn198Glu + Pro204Ser + Thr207Ser + Ala209Pro  
 + Leu211Asp  
 40 Pro195Ser + Gln200Asp + Thr202Asn + Tyr203Ser + Ala209Asp  
 + Asn212Ser  
 Gln200Asp + Pro204Ser + Gly205Pro + Tyr208His + Ala209Asp  
 + Leu211Asn  
 Pro204Glu + Ser206Asp + Tyr208Ile + Leu211Gly + Asn212Gln  
 45 + Thr214Asn  
 Gly196Ser + Asn198Ser + Val199Ala + Pro204Asp + Ser206Glu  
 + Tyr208Ser  
 Pro195Asn + Pro204Asp + Ser206Asp + Tyr208Ser + Leu211Ser  
 + Asn212Gln  
 50 Ala194Gly + Asn198Glu + Gln200Ser + Thr207Gly + Ala209Asn  
 + Asn212Glu  
 Asn198Glu + Gly205Gln + Ala209Pro + Leu211Cys + Asn212Asp  
 + Gly213Pro  
 Ala194Thr + Val197Thr + Asn198Gln + Pro204Gly + Gly205Asp

+ Thr207Asp  
Ala194Asn + Gly196Pro + Pro204Asp + Thr207Asp + Tyr208Cys  
+ Gly213Asn  
Ala194Thr + Pro195Gln + Gln200Ser + Pro204Glu + Thr207Asp  
5 + Gly213Pro  
Val197Pro + Asn198Glu + Gln200Asp + Leu211Ala + Asn212Ser  
+ Gly213Pro  
Asn198Asp + Gln200Glu + Tyr203Val + Leu211Thr + Asn212Ser  
+ Gly213Pro  
10 Pro195Gln + Asn198Asp + Val199Gln + Gln200Glu + Pro204Asn  
+ Ala209Pro  
Ala194Gly + Gly196Asn + Asn198Asp + Gln200Asp + Thr207Gly  
+ Asn212Ser  
Gln200Asp + Thr202Asn + Tyr203Ala + Thr207Gln + Leu211Glu  
+ Thr214Pro  
15 Val197Asp + Val199His + Tyr203Val + Tyr208His + Leu211Asp  
+ Gly213Pro  
Pro195Asn + Val197Gln + Val199Gln + Thr207Asp + Ala209Glu  
+ Gly213Ser  
20 Asn198Gln + Gln200Asn + Tyr203Ala + Thr207Asp + Tyr208Val  
+ Ala209Asp  
Val197Ala + Asn198Gln + Pro204Ser + Tyr208Thr + Ser210Glu  
+ Asn212Glu  
Gly196Asn + Gln200Asn + Pro204Gln + Ser210Asp + Asn212Glu  
+ Thr214Pro  
25 Pro195Gln + Val199Pro + Ser210Asp + Asn212Glu + Gly213Asn  
+ Thr214Gly  
Pro195Gly + Gln200Ser + Thr207Pro + Tyr208Gln + Ser210Glu  
+ Asn212Glu  
30 Pro195Gly + Val199Cys + Thr202Asn + Tyr208His + Ser210Glu  
+ Asn212Asp  
Val199His + Thr202Gly + Pro204Gln + Ala209Thr + Ser210Asp  
+ Asn212Asp  
Asn198Gln + Thr202Pro + Ala209Ser + Ser210Glu + Asn212Glu  
+ Gly213Pro  
35 Ala194Ser + Val199Gln + Tyr208Gly + Ser210Asp + Leu211Gln  
+ Asn212Asp  
Ala194Asn + Val199Gly + Gly205Ser + Ala209His + Ser210Glu  
+ Asn212Glu  
40 Ala194Ser + Thr202Asn + Pro204Gly + Tyr208Glu + Ser210Glu  
+ Gly213Gln  
Pro195Gln + Val199Asn + Thr207Gly + Tyr208Asp + Ser210Asp  
+ Leu211Ile  
Pro195Gln + Gln200Asn + Tyr203Gln + Tyr208Glu + Ser210Glu  
+ Asn212Gln  
45 Ala194His + Tyr203Asn + Tyr208Asp + Ser210Asp + Leu211His  
+ Thr214Ser  
Gly196Asn + Val197Gln + Thr202Asn + Tyr208Glu + Ala209Asn  
+ Ser210Glu  
50 Ala194Asn + Gln200Ser + Tyr203Met + Ser206Glu + Tyr208Glu  
+ Leu211Val  
Asn198Ser + Pro204Gly + Ser206Glu + Tyr208Asp + Asn212Gln  
+ Thr214Gly  
Ala194Pro + Gln200Asn + Thr202Pro + Tyr203Met + Asn212Asp

+ Thr214Asp  
 Pro195Ser + Asn198Glu + Gln200Ser + Thr202Asn + Gly205Pro  
 + Gly213Asp  
 Pro195Ser + Asn198Glu + Val199Ala + Ala209Glu + Leu211Asn  
 5 + Thr214Gly  
 Pro195Gly + Asn198Glu + Gln200Asn + Thr202Pro + Tyr203Ile  
 + Ala209Glu  
 Val197Ser + Asn198Asp + Tyr203Pro + Tyr208Met + Ala209Asp  
 + Leu211Gln  
 10 Pro195Ser + Asn198Asp + Gln200Ser + Tyr208Met + Ala209Asp  
 + Leu211Met  
 Gly196Asn + Val197Asp + Val199Gln + Gln200Glu + Gly205Asn  
 + Gly213Pro  
 Gln200Glu + Thr202Gln + Tyr203Met + Ala209Asn + Leu211Ala  
 15 + Asn212Asp  
 Ala194Pro + Gly196Pro + Gln200Asp + Thr202Asn + Tyr208Ser  
 + Asn212Glu  
 Val197Gly + Val199Ser + Gln200Glu + Tyr203Gly + Asn212Glu  
 + Gly213Pro  
 20 Pro204Asp + Thr207Gln + Tyr208Leu + Ala209Asp + Asn212Ser  
 + Thr214Asn  
 Ala194Ser + Pro195Gln + Thr202Ser + Pro204Asp + Ala209Asp  
 + Thr214Asn  
 Pro195Asn + Thr202Ser + Tyr203Leu + Tyr208Asp + Ala209His  
 25 + Leu211Asp  
 Ala194Asn + Gly196Pro + Ser206Glu + Tyr208His + Ala209Asp  
 + Thr214Gly  
 Pro204Gln + Gly205Asn + Ser206Glu + Thr207Gly + Ala209Asp  
 + Leu211Cys  
 30 Val197Gln + Thr202Gln + Pro204Gly + Ser206Asp + Ala209Asp  
 + Thr214Ser  
 Ala194Gly + Val197Gln + Asn198Gln + Tyr203Val + Ser206Asp  
 + Ala209Asp  
 Gly196Ser + Val197Asn + Gln200Asp + Thr202Pro + Thr207Asp  
 35 + Asn212Gln  
 Ala194Gly + Gly196Ser + Ser210Asp + Leu211Ser + Asn212Gln  
 + Gly213Glu  
 Val197Thr + Tyr203His + Thr207Gln + Tyr208Pro + Ser210Asp  
 + Gly213Asp  
 40 Asn198Ser + Val199Cys + Tyr203Val + Gly205Ser + Ser210Asp  
 + Gly213Asp  
 Val197His + Val199Asn + Pro204Gln + Ser210Asp + Asn212Ser  
 + Gly213Asp  
 Gly196Ser + Val197Ala + Gln200Asn + Thr202Pro + Ser210Glu  
 45 + Gly213Glu  
 Asn198Glu + Tyr203Gly + Gly205Gln + Thr207Pro + Tyr208Val  
 + Thr214Glu  
 Ala194Gly + Gly196Asn + Asn198Asp + Tyr203Leu + Tyr208His  
 + Thr214Glu  
 50 Pro195Gly + Asn198Glu + Val199Cys + Ala209Gln + Asn212Gln  
 + Thr214Asp  
 Asn198Asp + Val199Asn + Thr202Asn + Pro204Gln + Asn212Gln  
 + Thr214Asp  
 Val199Pro + Gln200Asp + Tyr203Asn + Leu211Pro + Gly213Glu



+ Thr214Asn  
 Pro195Ser + Gly196Pro + Val199Ala + Gln200Asp + Asn212Gln  
 + Gly213Asp  
 Ala194Thr + Pro195Gly + Gly196Pro + Gln200Glu + Gly205Asn  
 5 + Gly213Glu  
 Val199His + Gln200Glu + Pro204Ser + Leu211His + Gly213Glu  
 + Thr214Gln  
 Asn198Asp + Gln200Ser + Gly205Ser + Tyr208Asp + Leu211Ala  
 + Asn212Ser  
 10 Ala194Ser + Asn198Asp + Thr202Ser + Tyr208Asp + Ala209Pro  
 + Gly213Gln  
 Gln200Asn + Tyr203Pro + Pro204Glu + Gly205Pro + Ser210Asp  
 + Leu211Pro  
 Val197Ser + Tyr203Pro + Pro204Asp + Ala209Gly + Ser210Asp  
 15 + Thr214Asn  
 Gly196Asn + Pro204Glu + Ser210Glu + Leu211Pro + Asn212Ser  
 + Thr214Gly

TABLE 29

	Loop 6 - Heptuple Mutation Variants
20	Pro195Ser + Asn198Ser + Val199Met + Ser206Glu + Thr207Ser + Gly213Asn + Thr214Gln
	Ala194Gln + Pro195Asn + Val197Ser + Thr202Ser + Tyr203Ser + Ala209Ser + Asn212Asp
25	Val197Asp + Gln200Asn + Thr202Pro + Gly205Gln + Thr207Gly + Tyr208Met + Leu211Val
	Ala194Ser + Pro195Gly + Asn198Ser + Thr202Pro + Ser206Glu + Tyr208Ala + Thr214Gln
30	Ala194Pro + Pro195Asn + Thr202Gly + Tyr203Thr + Ser206Glu + Leu211Ser + Thr214Pro
	Gly196Gln + Val197Thr + Gln200Asn + Tyr203Cys + Gly205Ser + Ser210Glu + Leu211Val
	Ala194His + Val199Ser + Thr202Gly + Thr207Asn + Leu211Met + Gly213Glu + Thr214Pro
35	Val199Gly + Tyr203Cys + Pro204Gly + Thr207Asn + Ala209His + Asn212Asp + Gly213Asn
	Ala194Gln + Gly196Pro + Tyr203Ser + Pro204Ser + Tyr208Ser + Leu211Glu + Thr214Pro
	Gly196Gln + Gly205Asn + Thr207Asp + Tyr208Met + Asn212Gln + Gly213Ser + Thr214Pro
40	Gly196Gln + Val199Cys + Ser206Glu + Thr207Pro + Ala209Pro + Leu211Ile + Gly213Ser
	Thr202Pro + Tyr203Val + Pro204Asn + Ser206Asp + Asn212Ser + Gly213Ser + Thr214Gly
45	Ala194Thr + Pro195Asn + Gly196Pro + Gln200Asn + Pro204Ser + Asn212Glu + Gly213Gln
	Asn198Gln + Gln200Ser + Pro204Gln + Gly205Asn + Thr207Glu + Asn212Ser + Gly213Ser
	Ala194Ser + Gly196Ser + Asn198Ser + Tyr203Gly + Gly205Pro + Ser206Glu + Gly213Pro
50	Ala194Thr + Gly196Gln + Val197Pro + Thr202Gln + Pro204Ser + Ala209Gly + Asn212Glu

Pro195Gln + Gly196Pro + Asn198Gln + Pro204Ser + Gly205Glu  
 + Tyr208Gln + Gly213Asn  
 Val199Thr + Tyr203Gln + Pro204Gln + Gly205Asn + Thr207Glu  
 + Leu211Thr + Thr214Gln  
 5 Val197Thr + Asn198Glu + Thr202Gly + Gly205Pro + Thr207Pro  
 + Tyr208Met + Gly213Gln  
 Pro195Gly + Gly196Ser + Thr202Gly + Tyr203Gln + Pro204Gln  
 + Tyr208Pro + Thr214Gln  
 Pro195Asn + Asn198Asp + Gln200Ser + Pro204Gly + Gly205Gln  
 10 + Tyr208Gly + Ala209Asn  
 Pro195Asn + Val199His + Pro204Asn + Ala209Pro + Leu211Thr  
 + Asn212Ser + Thr214Pro  
 Asn198Gln + Val199Thr + Gln200Asp + Pro204Asn + Leu211Val  
 + Asn212Ser + Gly213Gln  
 15 Pro195Ser + Gly196Ser + Val197Met + Val199Gly + Gly205Pro  
 + Ser210Glu + Asn212Gln  
 Ala194Thr + Pro195Gln + Asn198Gln + Thr202Asn + Thr207Asp  
 + Tyr208Thr + Gly213Ser  
 Pro195Gln + Gly196Asn + Gly205Glu + Thr207Pro + Tyr208Asn  
 20 + Ala209His + Asn212Ser  
 Asn198Gln + Thr202Pro + Thr207Pro + Leu211Pro + Asn212Glu  
 + Gly213Asn + Thr214Ser  
 Ala194Pro + Val197Met + Pro204Ser + Thr207Gln + Tyr208Asn  
 + Ala209Gln + Thr214Gly  
 25 Ala194Asn + Val197Cys + Pro204Asn + Tyr208Thr + Ala209Pro  
 + Ser210Glu + Leu211Ser  
 Gly196Asn + Val197Gly + Tyr203Pro + Gly205Asp + Leu211Ala  
 + Gly213Asn + Thr214Ser  
 Ala194Thr + Pro195Asn + Tyr203Pro + Gly205Ser + Ser210Glu  
 30 + Leu211Ala + Gly213Pro  
 Ala194His + Gly196Asn + Val197Met + Asn198Ser + Ser206Asp  
 + Leu211Asn + Asn212Ser  
 Ala194Thr + Gly196Ser + Val199Ser + Thr202Asn + Ala209Pro  
 + Leu211Pro + Gly213Gln  
 35 Gly196Gln + Val199His + Thr202Pro + Tyr203Thr + Pro204Asp  
 + Gly205Ser + Ala209Asn  
 Val197Cys + Val199Gly + Thr202Gln + Tyr203His + Gly205Glu  
 + Tyr208Ile + Thr214Gln  
 Ala194Gly + Val197Cys + Asn198Asp + Gln200Ser + Tyr203Leu  
 40 + Ala209Gln + Asn212Gln  
 Ala194Gly + Tyr203Cys + Gly205Gln + Ser206Glu + Tyr208Met  
 + Leu211Cys + Thr214Gly  
 Gly196Ser + Val199Gln + Thr202Ser + Tyr203His + Pro204Gln  
 + Tyr208Leu + Gly213Pro  
 45 Asn198Gln + Thr202Asn + Tyr203Ala + Pro204Glu + Tyr208Gly  
 + Ala209Gln + Thr214Gln  
 Ala194Asn + Pro195Gln + Pro204Gly + Ser206Glu + Thr207Ser  
 + Tyr208Ala + Asn212Ser  
 Ala194Pro + Pro195Gln + Val197Ser + Asn198Ser + Gln200Glu  
 50 + Thr202Ser + Gly205Ser  
 Pro195Ser + Gly196Ser + Val199Gly + Thr202Gly + Pro204Glu  
 + Leu211Ala + Thr214Asn  
 Ala194Thr + Gly196Pro + Asn198Ser + Pro204Asn + Tyr208Gly  
 + Ser210Asp + Thr214Asn

Ala194Pro + Val199Asn + Thr202Gln + Pro204Gln + Gly205Asp  
+ Thr207Ser + Ala209Pro  
Ala194Pro + Pro195Gly + Val197Cys + Val199Gln + Tyr203Cys  
+ Tyr208His + Gly213Glu  
5 Val197Asp + Val199Ser + Gly205Pro + Thr207Gly + Leu211Thr  
+ Asn212Gln + Gly213Ser  
Ala194Pro + Pro195Ser + Gln200Asn + Thr202Pro + Tyr208His  
+ Gly213Asp + Thr214Gly  
Ala194Thr + Val197Asn + Val199Pro + Gln200Asp + Thr202Ser  
10 + Thr207Asn + Tyr208Leu  
Pro195Ser + Gly196Gln + Val197Ala + Thr202Gln + Tyr203Gly  
+ Tyr208Cys + Gly213Asn  
Ala194Gln + Val199Gln + Thr202Gly + Pro204Asn + Gly205Glu  
+ Ala209His + Leu211His  
15 Pro195Asn + Val199Thr + Tyr203Gly + Gly205Gln + Ser206Asp  
+ Asn212Ser + Gly213Pro  
Ala194Gln + Pro195Ser + Gly196Gln + Thr202Gln + Ala209His  
+ Asn212Ser + Thr214Glu  
Gly196Pro + Val197Cys + Tyr203Pro + Gly205Ser + Ser206Asp  
20 + Leu211Ile + Thr214Gly  
Gly196Asn + Val197Gly + Val199His + Thr202Ser + Ala209His  
+ Asn212Glu + Thr214Gln  
Gly196Gln + Gln200Ser + Thr202Gln + Gly205Gln + Ala209Ser  
+ Gly213Asp + Thr214Glu  
25 Val199Gly + Gln200Asn + Thr202Gln + Gly205Ser + Ala209Glu  
+ Ser210Asp + Leu211Asn  
Val199Pro + Gln200Asn + Tyr203Val + Ala209Asp + Ser210Asp  
+ Leu211Ser + Gly213Pro  
Ala194Gly + Tyr203Val + Gly205Asn + Thr207Gly + Ala209Glu  
30 + Ser210Asp + Leu211Pro  
Pro195Gln + Thr202Gly + Tyr203Val + Ala209Glu + Ser210Glu  
+ Gly213Ser + Thr214Gln  
Pro195Asn + Val197Ser + Tyr208Thr + Ala209Glu + Ser210Asp  
+ Asn212Ser + Thr214Gln  
35 Ala194Asn + Gly196Ser + Val199Gly + Thr202Pro + Tyr203Leu  
+ Asn212Glu + Gly213Asp  
Val197Cys + Asn198Ser + Gln200Ser + Thr202Asn + Thr207Gln  
+ Asn212Asp + Gly213Asp  
Gly196Pro + Thr202Asn + Pro204Asp + Gly205Glu + Ala209His  
40 + Leu211His + Gly213Ser  
Pro195Gly + Gly196Gln + Tyr203Thr + Thr207Asn + Ser210Asp  
+ Leu211Asp + Asn212Ser  
Ala194Gly + Gly196Pro + Thr202Gln + Tyr203Val + Gly205Ser  
+ Ser210Asp + Leu211Asp  
45 Ala194His + Val199Cys + Tyr203Ala + Tyr208Gly + Ser210Asp  
+ Leu211Asp + Gly213Pro  
Ala194His + Gly196Ser + Val197Cys + Val199Ser + Gly205Glu  
+ Ser206Glu + Tyr208Ser  
Ala194His + Gly196Ser + Val197Thr + Val199Cys + Gly205Asp  
50 + Ser206Glu + Thr214Gln  
Thr202Asn + Tyr203Thr + Gly205Glu + Ser206Asp + Ala209Gln  
+ Leu211Thr + Thr214Gly  
Ala194Pro + Val197Met + Asn198Ser + Val199Asn + Thr202Ser  
+ Ser206Asp + Thr207Glu

Pro195Asn + Val199Met + Thr202Gly + Ser206Glu + Thr207Asp  
+ Tyr208Thr + Asn212Ser  
Pro195Gly + Gly196Asn + Val199Gln + Tyr203His + Ser206Glu  
+ Thr207Asp + Asn212Gln  
5 Pro195Asn + Val199Ala + Gly205Pro + Ser206Glu + Thr207Asp  
+ Tyr208Gly + Asn212Ser  
Pro195Asn + Gly196Ser + Val197Ala + Pro204Ser + Ser206Glu  
+ Thr207Asp + Asn212Gln  
Pro195Asn + Val197Thr + Ser206Asp + Thr207Asp + Leu211Thr  
10 + Gly213Ser + Thr214Ser  
Ala194Thr + Val197Cys + Asn198Ser + Thr207Asp + Tyr208Asp  
+ Asn212Ser + Gly213Asn  
Ala194Thr + Gln200Asp + Thr202Gln + Ala209His + Ser210Asp  
+ Leu211Gly + Thr214Gly  
15 Pro195Gln + Gln200Asp + Tyr203Ala + Gly205Asn + Tyr208Ser  
+ Ser210Asp + Gly213Asn  
Ala194Thr + Val199Pro + Gln200Glu + Thr207Gly + Ser210Glu  
+ Leu211Ser + Gly213Asn  
Val197Ser + Gln200Glu + Tyr203Leu + Pro204Asn + Ala209Gly  
20 + Ser210Asp + Leu211Asn  
Gly196Gln + Asn198Gln + Val199Pro + Gln200Asp + Thr202Gly  
+ Pro204Gly + Ser210Asp  
Asn198Gln + Val199Cys + Gln200Glu + Gly205Gln + Ser210Asp  
+ Gly213Ser + Thr214Gly  
25 Asn198Gln + Gln200Glu + Gly205Pro + Thr207Ser + Ala209Asn  
+ Ser210Glu + Thr214Asn  
Gln200Glu + Thr202Asn + Thr207Ser + Tyr208Pro + Ser210Glu  
+ Gly213Gln + Thr214Gly  
Ala194Pro + Pro195Ser + Gly196Asn + Val197Asp + Val199Thr  
30 + Pro204Gly + Asn212Glu  
Gly196Asn + Asn198Glu + Tyr203Gly + Pro204Gln + Ser210Asp  
+ Leu211Val + Asn212Ser  
Gly196Pro + Val197Gly + Asn198Asp + Thr202Pro + Tyr203Val  
+ Thr207Asn + Ser210Glu  
35 Pro195Asn + Gly196Pro + Asn198Asp + Thr202Ser + Pro204Gln  
+ Ser210Asp + Gly213Gln  
Pro195Ser + Val197Pro + Asn198Glu + Val199Met + Thr207Ser  
+ Ser210Asp + Thr214Gln  
Ala194Ser + Gly196Asn + Val197Met + Asn198Asp + Val199Thr  
40 + Tyr208Met + Leu211Glu  
Asn198Asp + Val199Gln + Pro204Ser + Leu211Asp + Asn212Ser  
+ Gly213Pro + Thr214Asn  
Pro195Gln + Asn198Asp + Thr202Pro + Gly205Gln + Tyr208Met  
+ Leu211Glu + Gly213Asn  
45 Gly196Pro + Gln200Asp + Thr202Ser + Tyr203Thr + Tyr208His  
+ Ala209Glu + Leu211Ile  
Ala194Thr + Gln200Asn + Thr202Pro + Pro204Asp + Ser206Glu  
+ Thr207Asn + Ala209Ser  
Gly196Pro + Pro204Asp + Gly205Ser + Ser206Asp + Tyr208Gly  
50 + Gly213Gln + Thr214Gly  
Pro195Gln + Val197Gln + Gln200Ser + Pro204Glu + Ser206Asp  
+ Ala209Gly + Thr214Gln  
Ala194Thr + Pro195Gly + Val197Pro + Pro204Asp + Ser206Glu  
+ Leu211Val + Gly213Ser

Pro195Asn + Gly196Ser + Pro204Glu + Ser206Asp + Thr207Gly  
 + Tyr208Val + Ala209Thr  
 Pro195Asn + Gly196Pro + Val197Gln + Asn198Asp + Tyr203Gln  
 + Tyr208Asn + Asn212Asp  
 5 Gly196Gln + Val197Thr + Asn198Asp + Val199Gly + Thr202Pro  
 + Tyr208Asn + Asn212Glu  
 Pro195Asn + Asn198Asp + Val199Asn + Thr202Gln + Ala209Pro  
 + Asn212Asp + Gly213Gln  
 Ala194Gly + Val197Met + Asn198Asp + Gln200Glu + Thr202Asn  
 10 + Pro204Asn + Tyr208Asn  
 Pro195Ser + Val197Gln + Asn198Glu + Val199Cys + Gln200Asp  
 + Gly205Ser + Ala209Pro  
 Pro195Gln + Gly196Pro + Asn198Glu + Val199Ser + Gln200Asp  
 + Gly205Ser + Thr214Asn  
 15 Ala194Gln + Pro195Ser + Asn198Glu + Gln200Asp + Thr207Gln  
 + Tyr208Leu + Asn212Gln  
 Ala194Pro + Pro195Asn + Gln200Glu + Pro204Gln + Thr207Gln  
 + Tyr208Asp + Gly213Gln  
 Val199Thr + Gln200Asp + Tyr208Glu + Ala209Thr + Leu211Ser  
 20 + Asn212Ser + Gly213Asn  
 Gly196Pro + Val197Glu + Asn198Gln + Val199Met + Tyr208Met  
 + Gly213Asp + Thr214Asn  
 Pro195Gly + Val197Glu + Val199Thr + Gln200Ser + Gly205Asn  
 + Ala209Gly + Leu211Glu  
 25 Val197Met + Val199Cys + Gly205Pro + Tyr208Ile + Leu211Asp  
 + Asn212Ser + Gly213Asp  
 Pro195Gln + Asn198Ser + Val199Gln + Pro204Ser + Ser210Asp  
 + Leu211Cys + Asn212Glu  
 Pro195Ser + Gly196Pro + Asn198Gln + Pro204Gln + Ala209Thr  
 30 + Ser210Glu + Asn212Glu  
 Ala194Gly + Asn198Gln + Val199Gln + Thr207Ser + Ser210Asp  
 + Leu211Gly + Asn212Asp  
 Ala194Gln + Val197Gly + Thr202Ser + Pro204Ser + Ser210Glu  
 + Asn212Glu + Gly213Ser  
 35 Ala194Asn + Asn198Ser + Thr202Pro + Ser210Glu + Leu211Cys  
 + Asn212Asp + Gly213Ser  
 Pro195Gly + Val197Thr + Thr202Asn + Gly205Ser + Ser210Asp  
 + Asn212Glu + Gly213Ser  
 Pro195Gln + Gly205Gln + Ala209Gln + Ser210Asp + Leu211Asn  
 40 + Asn212Glu + Thr214Gln  
 Val199His + Pro204Asn + Tyr208Ile + Ala209Thr + Ser210Glu  
 + Leu211Gln + Asn212Asp  
 Val197Asn + Asn198Gln + Tyr203Pro + Pro204Asn + Ser210Glu  
 + Asn212Glu + Thr214Gln  
 45 Ala194Pro + Gly196Ser + Gln200Ser + Gly205Gln + Tyr208Gly  
 + Ser210Glu + Asn212Glu  
 Pro195Ser + Val197Gly + Asn198Gln + Val199Gln + Gln200Ser  
 + Ser210Glu + Asn212Asp  
 Asn198Gln + Gln200Asn + Thr207Gly + Tyr208Glu + Ser210Glu  
 50 + Asn212Ser + Thr214Asn  
 Pro195Gly + Thr202Ser + Tyr208Glu + Ala209Asn + Ser210Glu  
 + Asn212Gln + Thr214Asn  
 Val199Thr + Thr202Ser + Gly205Pro + Ser206Glu + Tyr208Glu  
 + Leu211His + Asn212Ser

- Ala194Gln + Gly196Pro + Asn198Gln + Tyr203Ile + Ser206Asp  
+ Tyr208Asp + Gly213Gln  
Ala194Ser + Pro195Ser + Thr202Ser + Gly205Gln + Ser206Asp  
+ Tyr208Asp + Asn212Gln  
5 Pro195Gly + Val197His + Tyr203Gln + Thr207Pro + Asn212Asp  
+ Gly213Ser + Thr214Asp  
Pro195Gly + Val197Met + Thr202Asn + Tyr208Met + Leu211Thr  
+ Asn212Glu + Thr214Asp  
Val197Ser + Asn198Gln + Thr202Ser + Pro204Glu + Thr207Ser  
10 + Tyr208Asp + Ala209Ser  
Ala194Pro + Val199Asn + Pro204Asp + Tyr208Glu + Leu211Gly  
+ Asn212Gln + Gly213Ser  
Pro195Gln + Val197Glu + Val199Gly + Gln200Ser + Thr207Ser  
+ Ser210Glu + Leu211Asn  
15 Pro195Ser + Val197Asp + Val199Cys + Pro204Gly + Tyr208Ser  
+ Ser210Glu + Asn212Ser  
Ala194Gln + Val197Asp + Asn198Ser + Thr202Asn + Thr207Asn  
+ Ser210Glu + Leu211His  
Val197Asp + Gln200Asn + Tyr203Thr + Tyr208Val + Ser210Asp  
20 + Asn212Ser + Thr214Ser  
Ala194Pro + Val197Asp + Gln200Ser + Thr202Gly + Leu211Cys  
+ Gly213Asn + Thr214Glu  
Pro195Ser + Val197Glu + Asn198Gln + Tyr208Ile + Ala209His  
+ Leu211Val + Thr214Glu  
25 Pro195Gly + Val197Asp + Gln200Ser + Thr202Pro + Ala209Thr  
+ Gly213Gln + Thr214Asp  
Pro195Asn + Gly196Pro + Asn198Asp + Pro204Asn + Gly205Asn  
+ Ala209Asn + Gly213Asp  
Ala194Pro + Asn198Asp + Val199Gln + Gln200Ser + Thr207Pro  
30 + Ala209Asp + Gly213Asn  
Val197Asp + Val199His + Gln200Glu + Thr202Pro + Tyr203Ile  
+ Thr207Gly + Thr214Gly  
Val197Glu + Gln200Asp + Tyr203Ile + Pro204Gln + Ala209Gly  
+ Leu211Ile + Thr214Pro  
35 Pro195Asn + Gly196Ser + Val199Met + Gln200Asp + Tyr203Ala  
+ Asn212Asp + Gly213Asn  
Ala194Thr + Gly196Gln + Gln200Glu + Tyr203Val + Tyr208Ala  
+ Leu211Ala + Asn212Glu  
Val197Asn + Asn198Gln + Pro204Asp + Tyr208Met + Ala209Glu  
40 + Asn212Ser + Gly213Asn  
Pro195Asn + Thr202Gly + Tyr203Asn + Pro204Glu + Gly205Asn  
+ Thr207Ser + Ala209Glu  
Ala194Pro + Gly196Pro + Tyr203Ala + Thr207Gln + Leu211Glu  
+ Gly213Pro + Thr214Glu  
45 Asn198Gln + Val199Thr + Thr202Pro + Pro204Gln + Ala209Thr  
+ Leu211Glu + Thr214Asp  
Ala194His + Val199Cys + Gln200Glu + Tyr203Ile + Gly205Asn  
+ Thr207Glu + Tyr208Met  
Val199Pro + Gln200Asp + Pro204Gln + Gly205Asn + Thr207Asp  
50 + Tyr208Cys + Thr214Pro  
Ala194His + Pro195Gln + Gly196Gln + Val199His + Gln200Glu  
+ Thr207Glu + Asn212Ser  
Ala194Gly + Asn198Gln + Gln200Asn + Tyr203Leu + Ser210Asp  
+ Leu211Gly + Gly213Glu

Asn198Gln + Gln200Asn + Thr202Ser + Ser210Asp + Leu211Ser  
+ Gly213Glu + Thr214Asn  
Pro195Asn + Val197Met + Tyr203Gly + Gly205Pro + Tyr208His  
+ Ser210Asp + Gly213Asp  
5 Pro195Ser + Gln200Asn + Thr207Pro + Ser210Glu + Leu211Met  
+ Gly213Glu + Thr214Gln  
Val197Gln + Val199Ser + Thr202Asn + Tyr208Gly + Ala209Gly  
+ Ser210Glu + Gly213Asp  
Gln200Asn + Pro204Gln + Thr207Glu + Ala209Ser + Ser210Asp  
10 + Leu211Thr + Gly213Asn  
Ala194Gln + Thr202Gly + Tyr203Asn + Thr207Asp + Ser210Asp  
+ Leu211Cys + Gly213Pro  
Pro195Gln + Val199Gln + Gly205Gln + Thr207Glu + Tyr208Ile  
+ Ser210Asp + Thr214Gly  
15 Pro195Gly + Val197His + Val199Met + Tyr203Thr + Thr207Glu  
+ Ser210Asp + Asn212Gln  
Ala194Gln + Pro195Gln + Asn198Ser + Val199Gln + Pro204Gln  
+ Thr207Glu + Ser210Glu  
Val197Pro + Asn198Glu + Val199Thr + Pro204Asn + Leu211Cys  
20 + Asn212Ser + Thr214Asp  
Ala194Ser + Val199Thr + Gln200Glu + Tyr203Cys + Tyr208Gly  
+ Leu211Gly + Gly213Glu  
Val199Pro + Gln200Asp + Tyr203Asn + Pro204Gly + Leu211Pro  
+ Gly213Glu + Thr214Asn  
25 Ala194Gln + Asn198Glu + Thr207Ser + Tyr208Glu + Asn212Gln  
+ Gly213Gln + Thr214Gln  
Pro195Gln + Asn198Asp + Gln200Ser + Gly205Ser + Tyr208Asp  
+ Leu211Ala + Asn212Ser  
Gly196Pro + Val197Asp + Asn198Gln + Val199Ala + Pro204Gln  
30 + Ala209Asp + Gly213Asn  
Pro195Ser + Asn198Gln + Val199Gly + Pro204Glu + Ala209Gly  
+ Ser210Asp + Asn212Gln  
Pro195Ser + Gln200Ser + Pro204Glu + Thr207Pro + Ala209Gln  
+ Ser210Asp + Asn212Gln  
35 Ala194His + Gly196Ser + Val197Gly + Val199Asn + Gln200Glu  
+ Pro204Glu + Ala209Thr  
Gly196Pro + Asn198Ser + Pro204Asn + Tyr208Gly + Ala209Asp  
+ Leu211Thr + Gly213Asp  
Gly196Pro + Val199Thr + Gln200Asp + Thr202Asn + Tyr203Asn  
40 + Ser206Glu + Ala209Ser  
Pro195Ser + Gly196Pro + Val199Pro + Gln200Glu + Pro204Gln  
+ Ser206Asp + Leu211Ala  
Pro195Ser + Gln200Asp + Thr202Gln + Pro204Gln + Ser206Glu  
+ Tyr208Gly + Ala209Gly  
45 Gly196Asn + Val199Ser + Gln200Glu + Thr202Pro + Ala209Thr  
+ Leu211Cys + Thr214Asp  
Val197Asn + Gln200Glu + Tyr203Gln + Gly205Pro + Ala209Thr  
+ Leu211Val + Thr214Glu  
Pro195Gly + Tyr203Gly + Ser206Glu + Tyr208Gly + Ala209Gln  
50 + Ser210Glu + Leu211Ile  
Gly196Asn + Val197His + Val199Ala + Pro204Asn + Ser206Asp  
+ Ser210Asp + Leu211Thr  
Ala194Gln + Val197Ser + Val199Pro + Ser206Asp + Ser210Glu  
+ Leu211Pro + Thr214Gly

Ala194His + Gln200Ser + Thr202Asn + Ser206Glu + Tyr208Cys  
 + Ser210Asp + Asn212Ser  
 Val197Thr + Thr202Ser + Pro204Gly + Gly205Asn + Ser206Asp  
 + Ser210Glu + Leu211Pro

5

TABLE 30

## Loop 6 - Octuple Mutation Variants

	Pro195Ser + Val197Ser + Asn198Ser + Val199Gly + Tyr203Thr + Thr207Asn + Tyr208Met + Asn212Glu
10	Pro195Gln + Gly196Gln + Val199Gln + Tyr203Gln + Tyr208Met + Ala209Gly + Leu211Val + Gly213Asp
	Pro195Gln + Asn198Gln + Val199His + Pro204Gly + Ser206Glu + Thr207Gln + Tyr208Ser + Leu211Gln
15	Ala194Thr + Val197Met + Val199His + Thr202Ser + Tyr203Ile + Thr207Gln + Tyr208Pro + Leu211Gly
	Pro195Gln + Val197Asn + Val199Cys + Tyr203Met + Pro204Asn + Gly205Glu + Thr207Pro + Tyr208Ser
	Gly196Ser + Gln200Asn + Pro204Asp + Gly205Ser + Thr207Pro + Tyr208Met + Gly213Gln + Thr214Ser
20	Val197Gln + Gln200Asn + Tyr203His + Pro204Asp + Gly205Ser + Tyr208Thr + Ala209Gln + Leu211Pro
	Pro195Gly + Gly196Ser + Val197Pro + Val199Ser + Thr202Asn + Ala209Glu + Leu211Ser + Gly213Pro
25	Ala194His + Gly196Ser + Val197Asn + Asn198Ser + Tyr203Leu + Pro204Asn + Thr207Ser + Ala209Thr
	Ala194Pro + Gly196Pro + Val199Met + Gln200Asp + Thr202Asn + Thr207Asn + Ala209Pro + Gly213Pro
	Pro195Asn + Gly196Pro + Val199Thr + Thr202Gln + Thr207Gly + Tyr208Ser + Ser210Asp + Asn212Ser
30	Ala194Thr + Pro195Asn + Gly196Gln + Thr202Pro + Pro204Ser + Gly205Pro + Tyr208Gln + Leu211Asp
	Ala194Gly + Pro195Ser + Val197Ala + Thr202Gly + Tyr203Gly + Pro204Gln + Gly205Ser + Leu211Glu
	Pro195Asn + Val197Glu + Val199Thr + Gln200Asn + Thr202Pro + Tyr203Ile + Pro204Ser + Leu211Cys
35	Ala194His + Pro195Gln + Val199Cys + Gln200Asp + Tyr203Ala + Thr207Ser + Gly213Gln + Thr214Pro
	Ala194Gln + Gly196Gln + Val199His + Thr202Pro + Tyr203Thr + Pro204Asp + Gly205Ser + Ala209Asn
40	Pro195Ser + Gly196Ser + Gln200Asn + Tyr203His + Thr207Pro + Leu211Met + Gly213Glu + Thr214Gln
	Val197Cys + Asn198Ser + Val199Ala + Gln200Asp + Tyr203His + Leu211Asn + Asn212Gln + Gly213Asn
	Ala194Gln + Gly196Pro + Asn198Gln + Val199His + Tyr203Val + Thr207Ser + Ala209Ser + Leu211Asn
45	Pro195Gly + Gly196Ser + Val197Pro + Thr207Gly + Leu211Thr + Asn212Ser + Gly213Asn + Thr214Glu
	Val197Asn + Thr202Gly + Tyr203Ala + Gly205Gln + Tyr208Ile + Ala209Thr + Ser210Asp + Leu211Gly
50	Ala194Asn + Asn198Gln + Val199Ser + Thr202Gly + Tyr203Ile + Ala209Thr + Ser210Asp + Thr214Ser
	Pro195Ser + Gly196Asn + Val197Asn + Asn198Ser + Thr202Asn



+ Tyr203Cys + Tyr208Asn + Leu211Thr  
 Ala194Gly + Pro195Ser + Gly196Gln + Val199Ala + Thr202Pro  
 + Thr207Ser + Asn212Ser + Thr214Asp  
 Val197Cys + Val199Pro + Thr202Pro + Tyr203Ser + Ser206Glu  
 5 + Thr207Gln + Ala209Gly + Thr214Gly  
 Ala194Gly + Pro195Gln + Gly196Gln + Asn198Ser + Val199Met  
 + Thr202Pro + Thr207Glu + Thr214Gly  
 Ala194His + Pro195Gln + Val197Gln + Asn198Gln + Tyr203Ser  
 + Ser206Glu + Tyr208Ala + Leu211Ala  
 10 Ala194Gly + Gly196Ser + Tyr203His + Pro204Asn + Ser206Asp  
 + Tyr208Ile + Leu211Gln + Asn212Ser  
 Gly196Pro + Gln200Ser + Tyr203Gln + Pro204Ser + Gly205Ser  
 + Tyr208Cys + Leu211Met + Gly213Gln  
 Ala194Asn + Tyr203Asn + Gly205Glu + Thr207Gln + Ala209Thr  
 15 + Leu211His + Gly213Pro + Thr214Gln  
 Ala194Ser + Pro195Asn + Gly196Asn + Val199Ser + Pro204Ser  
 + Gly205Asn + Ala209Asp + Thr214Gln  
 Pro195Asn + Gly196Gln + Thr202Pro + Gly205Ser + Ser206Glu  
 + Tyr208Ser + Ala209Pro + Gly213Gln  
 20 Ala194Thr + Pro195Gly + Val197Asp + Asn198Gln + Gly205Ser  
 + Tyr208Val + Asn212Gln + Gly213Ser  
 Pro195Gly + Asn198Ser + Tyr203Thr + Pro204Asn + Tyr208Pro  
 + Ala209Thr + Asn212Ser + Gly213Ser  
 Ala194Thr + Pro195Ser + Gly196Asn + Val197Met + Gln200Ser  
 25 + Thr202Asn + Tyr203His + Tyr208Gln  
 Ala194His + Asn198Ser + Tyr203Gln + Pro204Gln + Ala209Pro  
 + Leu211Asp + Asn212Glu + Gly213Asn  
 Ala194Asn + Pro195Ser + Val197Gly + Pro204Gly + Gly205Asn  
 + Ala209Asp + Ser210Asp + Thr214Gln  
 30 Gly196Gln + Val197Pro + Gln200Asn + Thr202Asn + Gly205Gln  
 + Ala209Glu + Ser210Asp + Thr214Pro  
 Val197Ser + Val199Ala + Thr202Ser + Tyr203Pro + Gly205Asn  
 + Tyr208Ile + Ala209Asp + Ser210Glu  
 Asn198Ser + Val199Met + Gln200Ser + Pro204Ser + Tyr208Ala  
 35 + Ala209Glu + Ser210Asp + Leu211Gly  
 Ala194Gly + Val197Asn + Asn198Ser + Gln200Ser + Tyr203Thr  
 + Ala209Asp + Ser210Asp + Thr214Pro  
 Ala194His + Pro195Ser + Asn198Ser + Tyr203Cys + Thr207Ser  
 + Ala209Asp + Ser210Glu + Gly213Ser  
 40 Pro195Ser + Val199Asn + Gln200Ser + Pro204Ser + Thr207Gly  
 + Ala209Asp + Ser210Asp + Asn212Ser  
 Ala194Gln + Gly196Pro + Val197Asp + Asn198Glu + Val199Ser  
 + Gly205Gln + Ala209Gly + Asn212Ser  
 Pro195Gln + Val197Asp + Asn198Glu + Val199Ala + Gln200Ser  
 45 + Thr202Ser + Thr207Gly + Tyr208Ala  
 Pro195Ser + Gly196Pro + Gln200Asn + Thr202Asn + Tyr203Met  
 + Thr207Gly + Asn212Asp + Gly213Asp  
 Gly196Asn + Thr202Pro + Pro204Gly + Thr207Pro + Leu211Cys  
 + Asn212Glu + Gly213Glu + Thr214Pro  
 50 Gly196Asn + Asn198Gln + Gln200Ser + Thr202Pro + Tyr203Cys  
 + Pro204Asn + Asn212Asp + Gly213Glu  
 Asn198Ser + Val199Gly + Tyr203Ala + Pro204Asp + Gly205Glu  
 + Tyr208Met + Ala209Ser + Leu211Pro  
 Val197Cys + Val199Asn + Thr202Gln + Pro204Asp + Gly205Asp

+ Ala209Gln + Leu211Gln + Asn212Ser  
Pro195Asn + Val199Thr + Thr202Gly + Ala209His + Ser210Glu  
+ Leu211Asp + Asn212Ser + Gly213Asn  
Gly196Asn + Val199His + Tyr203Ala + Gly205Asp + Ser206Asp  
5 + Thr207Gln + Tyr208Gln + Leu211Asn  
Ala194Gly + Gly196Gln + Val199Gln + Gln200Asn + Pro204Gly  
+ Gly205Asp + Ser206Asp + Thr214Ser  
Ala194Gln + Pro195Asn + Val197Ala + Gln200Asn + Tyr203Ser  
+ Gly205Asp + Ser206Asp + Tyr208Ala  
10 Ala194Gln + Val197Gln + Pro204Asn + Gly205Asp + Ser206Glu  
+ Tyr208Ala + Ala209His + Gly213Asn  
Pro195Ser + Gly196Asn + Val197Thr + Gln200Asn + Pro204Ser  
+ Gly205Glu + Ser206Glu + Tyr208Gly  
Val197Gly + Asn198Gln + Val199Met + Gln200Asn + Tyr203His  
15 + Ser206Asp + Thr207Glu + Thr214Pro  
Ala194Gln + Pro195Gly + Gly196Pro + Thr202Pro + Ser206Asp  
+ Thr207Asp + Tyr208Val + Ala209His  
Ala194Thr + Pro195Ser + Val197Gln + Asn198Gln + Thr202Ser  
+ Pro204Asn + Ser206Glu + Thr207Asp  
20 Ala194Gln + Val199Met + Thr202Ser + Tyr203Gly + Gly205Gln  
+ Ser206Asp + Thr207Glu + Gly213Ser  
Ala194Gly + Pro195Asn + Gly196Gln + Tyr203Ala + Ser206Asp  
+ Thr207Glu + Ala209Thr + Asn212Gln  
Gly196Ser + Gln200Ser + Tyr203Leu + Pro204Ser + Ser206Glu  
25 + Thr207Glu + Ala209Ser + Gly213Ser  
Ala194Ser + Pro195Asn + Thr202Gln + Ser206Glu + Thr207Glu  
+ Tyr208Gly + Asn212Ser + Thr214Gly  
Ala194His + Pro195Gln + Val197Ala + Thr202Asn + Tyr203Pro  
+ Gly205Pro + Tyr208Asp + Ala209Asp  
30 Ala194Gln + Pro195Gln + Val197Asn + Gln200Asp + Ala209Gly  
+ Ser210Glu + Leu211Ala + Thr214Asn  
Ala194Pro + Val199Met + Gln200Glu + Thr202Gly + Pro204Gln  
+ Gly205Asn + Tyr208Gln + Ser210Asp  
Gly196Pro + Val197Cys + Val199Asn + Gln200Glu + Gly205Pro  
35 + Tyr208Val + Ser210Glu + Thr214Pro  
Ala194Thr + Pro195Gly + Gly196Gln + Val197Asp + Val199His  
+ Gln200Ser + Thr202Ser + Asn212Asp  
Asn198Asp + Val199Pro + Gly205Asn + Thr207Gly + Tyr208Pro  
+ Ser210Asp + Leu211Gly + Gly213Gln  
40 Ala194Gln + Gly196Pro + Asn198Glu + Tyr203Gln + Thr207Gln  
+ Tyr208Pro + Ala209Pro + Ser210Glu  
Ala194Pro + Asn198Asp + Val199His + Gly205Asn + Ser210Glu  
+ Leu211Asn + Asn212Ser + Thr214Pro  
Asn198Glu + Val199Pro + Tyr203Asn + Thr207Pro + Ala209Asn  
45 + Ser210Glu + Asn212Ser + Gly213Gln  
Ala194Gly + Pro195Asn + Asn198Asp + Tyr203Ala + Gly205Gln  
+ Thr207Ser + Ser210Glu + Leu211Val  
Ala194Thr + Asn198Asp + Thr202Gly + Pro204Ser + Gly205Ser  
+ Thr207Asn + Ala209Pro + Ser210Glu  
50 Ala194Gln + Asn198Asp + Tyr203Ala + Thr207Gln + Tyr208Pro  
+ Ser210Glu + Leu211Pro + Asn212Ser  
Ala194Thr + Pro195Ser + Asn198Asp + Tyr203Cys + Pro204Asn  
+ Thr207Gln + Tyr208Gln + Ser210Glu  
Pro195Ser + Asn198Asp + Val199Ser + Gln200Ser + Pro204Ser

+ Ser210Glu + Leu211Gly + Asn212Ser  
 Ala194Gln + Pro195Ser + Gly196Pro + Gln200Glu + Pro204Asn  
 + Gly205Pro + Ala209Glu + Leu211Cys  
 Val199His + Gln200Ser + Pro204Asp + Gly205Ser + Ser206Asp  
 5 + Tyr208Gly + Gly213Gln + Thr214Gly  
 Gly196Asn + Asn198Ser + Thr202Ser + Pro204Asp + Gly205Ser  
 + Ser206Asp + Thr207Ser + Tyr208Val  
 Val197Ala + Gln200Asn + Pro204Glu + Gly205Ser + Ser206Glu  
 + Tyr208Val + Leu211Ser + Asn212Ser  
 10 Pro195Asn + Val197Thr + Thr202Ser + Tyr203Ile + Pro204Asp  
 + Ser206Asp + Thr207Gln + Ala209Gln  
 Pro195Gly + Val197Asn + Val199Ser + Gln200Asn + Tyr203Asn  
 + Pro204Glu + Ser206Glu + Leu211Met  
 Gly196Ser + Asn198Ser + Val199Met + Gln200Asn + Pro204Glu  
 15 + Ser206Asp + Leu211Val + Thr214Gln  
 Ala194Gln + Gly196Asn + Asn198Asp + Gln200Ser + Thr202Asn  
 + Pro204Asn + Asn212Glu + Thr214Asn  
 Pro195Gly + Asn198Glu + Val199Asn + Gln200Ser + Thr202Gly  
 + Thr207Asn + Ala209Thr + Asn212Asp  
 20 Pro195Asn + Gly196Ser + Asn198Glu + Thr207Ser + Tyr208Ser  
 + Ala209Pro + Asn212Asp + Thr214Asn  
 Ala194Pro + Val197Ser + Asn198Glu + Gln200Asn + Tyr203Cys  
 + Thr207Pro + Tyr208Thr + Asn212Asp  
 Pro195Gly + Gly196Ser + Thr202Asn + Pro204Asn + Gly205Glu  
 25 + Thr207Asp + Tyr208Thr + Asn212Ser  
 Val197Gln + Val199Asn + Thr202Pro + Gly205Glu + Thr207Glu  
 + Tyr208Ala + Leu211Ser + Gly213Asn  
 Ala194Ser + Pro195Ser + Val197Gly + Pro204Asp + Gly205Ser  
 + Thr207Glu + Tyr208Val + Leu211Gly  
 30 Ala194Gln + Pro195Gly + Gly196Asn + Val197Cys + Asn198Gln  
 + Pro204Glu + Thr207Asp + Gly213Gln  
 Pro195Gly + Asn198Asp + Gln200Glu + Tyr203Thr + Gly205Asn  
 + Leu211Cys + Asn212Ser + Gly213Gln  
 Ala194Gly + Pro195Asn + Asn198Glu + Gln200Glu + Thr202Gln  
 35 + Gly205Ser + Thr207Pro + Gly213Gln  
 Pro195Gly + Asn198Glu + Val199Gln + Gln200Asp + Thr202Gly  
 + Pro204Gln + Thr207Pro + Leu211Cys  
 Pro195Asn + Gly196Gln + Asn198Glu + Gln200Asp + Thr202Gly  
 + Tyr208Gln + Leu211Ser + Thr214Ser  
 40 Val197Asn + Asn198Ser + Gln200Glu + Thr202Asn + Tyr203Cys  
 + Pro204Ser + Gly205Pro + Tyr208Glu  
 Ala194His + Val199His + Tyr203Asn + Tyr208His + Ala209Glu  
 + Leu211Asp + Asn212Gln + Gly213Gln  
 Gly196Gln + Val197Ser + Gln200Glu + Tyr203Val + Thr207Pro  
 45 + Ala209Thr + Leu211Glu + Asn212Ser  
 Pro195Gln + Val199Gly + Gln200Asp + Pro204Asn + Gly205Asn  
 + Tyr208Ile + Ala209Pro + Leu211Glu  
 Pro195Gln + Asn198Ser + Val199Asn + Gln200Asp + Gly205Pro  
 + Tyr208Ser + Leu211Glu + Thr214Asn  
 50 Ala194His + Pro195Ser + Val197Glu + Val199Cys + Thr202Pro  
 + Leu211Glu + Asn212Gln + Thr214Asn  
 Ala194Ser + Pro195Gln + Asn198Ser + Gln200Asn + Gly205Gln  
 + Leu211Asp + Asn212Gln + Gly213Glu  
 Gly196Gln + Val199Gln + Tyr203Gly + Gly205Asn + Ala209His

+ Leu211Asp + Asn212Gln + Gly213Asp  
 Asn198Gln + Gln200Ser + Tyr203Leu + Gly205Asn + Thr207Glu  
 + Ala209Asp + Leu211Ser + Gly213Asn  
 Ala194Ser + Pro195Ser + Val199Gln + Gln200Ser + Gly205Gln  
 5 + Ser210Asp + Asn212Glu + Thr214Gln  
 Pro195Asn + Tyr203Cys + Pro204Gln + Tyr208Asn + Ala209His  
 + Ser210Asp + Asn212Asp + Thr214Gly  
 Gly196Asn + Val199Ser + Thr202Gln + Pro204Ser + Thr207Gly  
 + Ser210Glu + Leu211Thr + Asn212Asp  
 10 Gly196Gln + Val197Cys + Asn198Ser + Pro204Gln + Thr207Asn  
 + Ala209Gln + Ser210Asp + Asn212Asp  
 Pro195Gly + Gly196Pro + Val197Cys + Asn198Ser + Gln200Asn  
 + Tyr208His + Ser210Asp + Asn212Glu  
 Pro195Gln + Asn198Ser + Val199Gly + Thr202Pro + Thr207Ser  
 15 + Tyr208Ile + Ser210Asp + Asn212Asp  
 Ala194Asn + Val197Met + Val199Thr + Ala209Asn + Ser210Asp  
 + Leu211Val + Asn212Glu + Gly213Gln  
 Ala194Gln + Pro195Asn + Val199Asn + Tyr203Ser + Thr207Asn  
 + Ser210Asp + Leu211Gly + Asn212Asp  
 20 Val199Cys + Gln200Ser + Thr202Asn + Gly205Asn + Thr207Gly  
 + Tyr208Asn + Ser210Glu + Asn212Asp  
 Ala194Thr + Val199Cys + Gln200Ser + Gly205Ser + Ser206Glu  
 + Tyr208Asp + Leu211Ala + Gly213Asn  
 Ala194Pro + Val197Pro + Gln200Ser + Ser206Asp + Thr207Pro  
 25 + Tyr208Asp + Ala209Pro + Thr214Gly  
 Gly196Gln + Asn198Ser + Val199Met + Thr202Ser + Pro204Asn  
 + Ser206Glu + Tyr208Glu + Asn212Gln  
 Pro195Asn + Gly196Asn + Val199Ala + Gln200Asn + Ser206Asp  
 + Thr207Asn + Tyr208Asp + Ala209Ser  
 30 Gly196Asn + Tyr203Ala + Pro204Ser + Ser206Asp + Tyr208Asp  
 + Ala209Ser + Asn212Ser + Thr214Gln  
 Val197Gly + Asn198Ser + Tyr203Asn + Pro204Gly + Gly205Ser  
 + Thr207Pro + Asn212Asp + Thr214Asp  
 Ala194Gln + Val197Asp + Asn198Gln + Tyr203His + Ser210Glu  
 35 + Leu211Ala + Gly213Ser + Thr214Asn  
 Ala194Pro + Val197Glu + Val199His + Gln200Ser + Tyr203His  
 + Pro204Asn + Tyr208Thr + Thr214Glu  
 Gly196Asn + Asn198Asp + Val199Gln + Gln200Ser + Thr202Ser  
 + Pro204Ser + Leu211Cys + Gly213Glu  
 40 Asn198Asp + Tyr203Met + Pro204Gly + Thr207Asn + Tyr208Gln  
 + Ala209Glu + Asn212Ser + Thr214Ser  
 Asn198Glu + Val199Gly + Thr202Gly + Thr207Gln + Tyr208Cys  
 + Ala209Asp + Leu211Thr + Asn212Gln  
 Ala194His + Val197Asp + Asn198Gln + Gln200Glu + Thr202Asn  
 45 + Tyr208Pro + Leu211Ser + Asn212Ser  
 Pro195Gly + Gln200Asp + Tyr203Gly + Gly205Asn + Leu211Ser  
 + Asn212Glu + Gly213Gln + Thr214Gln  
 Ala194Asn + Gly196Ser + Val199Pro + Gln200Glu + Thr207Gly  
 + Tyr208Asn + Ala209Thr + Asn212Glu  
 50 Ala194Thr + Val199Asn + Gln200Glu + Tyr203Gln + Pro204Gly  
 + Ala209His + Asn212Glu + Thr214Ser  
 Ala194Asn + Gly196Pro + Val197Ser + Asn198Gln + Tyr203Pro  
 + Pro204Asp + Tyr208Asn + Ala209Asp  
 Pro195Ser + Val199Cys + Gln200Ser + Pro204Glu + Gly205Ser

+ Tyr208Ala + Ala209Glu + Asn212Gln  
 Asn198Gln + Val199His + Thr202Gly + Pro204Glu + Gly205Pro  
 + Tyr208Ala + Ala209Glu + Asn212Gln  
 Gly196Ser + Asn198Ser + Val199Gly + Gln200Ser + Gly205Ser  
 5 + Tyr208Asp + Leu211Asp + Thr214Ser  
 Pro195Gln + Gly196Asn + Val197Gly + Gln200Asn + Pro204Asn  
 + Thr207Gly + Ala209Asp + Asn212Glu  
 Ala194Ser + Gln200Ser + Gly205Ser + Ala209Asp + Leu211Gly  
 + Asn212Asp + Gly213Gln + Thr214Asn  
 10 Ala194Gln + Pro195Gln + Gly196Pro + Tyr203Leu + Pro204Ser  
 + Ser206Asp + Ala209Glu + Leu211Pro  
 Thr202Asn + Tyr203Ala + Pro204Ser + Ser206Asp + Tyr208Cys  
 + Ala209Glu + Leu211Cys + Asn212Ser  
 Gly196Ser + Val199His + Gln200Asn + Gly205Asn + Ser206Glu  
 15 + Ala209Asp + Leu211His + Asn212Ser  
 Ala194Asn + Val197Ala + Gln200Ser + Ser206Glu + Thr207Pro  
 + Ala209Glu + Asn212Gln + Gly213Asn  
 Pro195Gln + Val197Met + Val199Thr + Gln200Ser + Tyr203Ala  
 + Ala209Ser + Ser210Glu + Gly213Glu  
 20 Asn198Ser + Val199Asn + Ala209Pro + Ser210Glu + Leu211Gly  
 + Asn212Ser + Gly213Asp + Thr214Gly  
 Ala194Gly + Asn198Gln + Tyr203Ala + Gly205Asp + Tyr208Gln  
 + Ala209Asp + Leu211Ala + Asn212Gln  
 Ala194Gly + Gly196Gln + Thr202Gln + Tyr203Thr + Thr207Asp  
 25 + Tyr208Thr + Ser210Glu + Asn212Ser  
 Ala194Pro + Val197Thr + Tyr203Pro + Pro204Ser + Thr207Asp  
 + Ser210Asp + Leu211Pro + Thr214Ser  
 Pro195Asn + Val199Ala + Thr202Ser + Gly205Gln + Thr207Asp  
 + Ala209His + Ser210Glu + Gly213Asn  
 30 Pro195Gly + Gly196Ser + Val197Pro + Pro204Gly + Thr207Glu  
 + Tyr208Gln + Ala209His + Ser210Asp  
 Ala194Asn + Pro195Asn + Asn198Ser + Val199His + Thr207Asp  
 + Tyr208Val + Ala209Pro + Ser210Glu  
 Ala194Gly + Gly196Ser + Asn198Asp + Thr207Asn + Ala209Gln  
 35 + Leu211Pro + Gly213Gln + Thr214Asp  
 Ala194Gly + Pro195Ser + Val197His + Asn198Asp + Gln200Asn  
 + Pro204Ser + Asn212Ser + Thr214Asp  
 Val199Asn + Gln200Glu + Pro204Gly + Gly205Asn + Tyr208Ser  
 + Leu211Ala + Asn212Ser + Gly213Glu  
 40 Ala194Gln + Pro195Ser + Gly196Asn + Asn198Gln + Gln200Glu  
 + Tyr203Cys + Leu211Ser + Gly213Glu  
 Ala194Ser + Gly196Asn + Val197Thr + Asn198Gln + Gln200Glu  
 + Thr207Asn + Tyr208His + Gly213Asp  
 Ala194Gln + Val197Gln + Asn198Asp + Gln200Asn + Gly205Gln  
 45 + Tyr208Asp + Leu211Gly + Thr214Ser  
 Ala194Ser + Asn198Ser + Thr202Pro + Pro204Asp + Gly205Gln  
 + Thr207Asn + Ser210Asp + Asn212Ser  
 Pro195Gln + Gly196Asn + Pro204Glu + Gly205Asn + Thr207Asn  
 + Tyr208Ser + Ala209Pro + Ser210Asp  
 50 Ala194Thr + Gly196Pro + Val199Pro + Gln200Asn + Pro204Glu  
 + Ala209Thr + Ser210Asp + Leu211Ala  
 Ala194Ser + Gly196Gln + Val199Thr + Pro204Glu + Tyr208Ile  
 + Ala209Gln + Ser210Asp + Thr214Gly  
 Ala194His + Val197Ser + Val199Ser + Gln200Ser + Pro204Glu

+ Thr207Gly + Ala209Pro + Ser210Asp  
 Ala194Gln + Val197Ala + Asn198Ser + Pro204Asp + Tyr208Cys  
 + Ala209Gly + Ser210Asp + Asn212Ser  
 Gly196Ser + Val197Thr + Asn198Ser + Gln200Glu + Thr202Pro  
 5 + Pro204Glu + Ala209Thr + Gly213Ser  
 Pro195Asn + Val197Pro + Val199Gly + Gln200Asp + Pro204Glu  
 + Thr207Gly + Leu211Thr + Gly213Ser  
 Ala194Gln + Val197Asn + Gln200Ser + Thr202Gln + Thr207Glu  
 + Ala209Gly + Leu211Glu + Gly213Asn  
 10 Ala194Ser + Gly196Ser + Val197Ser + Asn198Gln + Gly205Gln  
 + Ser210Glu + Asn212Ser + Thr214Asp  
 Gly196Ser + Val199Cys + Gln200Ser + Thr207Pro + Tyr208Pro  
 + Ala209Gly + Ser210Asp + Thr214Asp  
 Val197Cys + Val199Gly + Tyr203Gly + Tyr208Met + Ala209Ser  
 15 + Ser210Glu + Leu211Ala + Thr214Glu  
 Ala194Pro + Pro195Ser + Gly196Ser + Val199Asn + Thr202Gly  
 + Ser210Asp + Asn212Gln + Thr214Glu  
 Ala194Asn + Gly196Asn + Gln200Ser + Pro204Asn + Gly205Ser  
 + Ala209Ser + Ser210Asp + Thr214Asp  
 20 Ala194His + Val197Cys + Tyr208Ile + Ser210Glu + Leu211Gly  
 + Asn212Ser + Gly213Asn + Thr214Asp  
 Gly196Gln + Gln200Asn + Thr202Pro + Tyr203Cys + Gly205Gln  
 + Tyr208Ser + Ser210Glu + Thr214Asp  
 Asn198Gln + Val199Pro + Gln200Glu + Thr202Pro + Pro204Ser  
 25 + Ser206Glu + Tyr208Gln + Thr214Asn  
 Pro195Gly + Gln200Asp + Tyr203Val + Ser206Asp + Tyr208Thr  
 + Leu211Gly + Asn212Gln + Gly213Asn  
 Gly196Gln + Gln200Glu + Thr202Gln + Tyr203Val + Ser206Glu  
 + Tyr208Val + Ala209Asn + Leu211Gly  
 30 Pro195Gly + Gln200Glu + Thr202Pro + Gly205Ser + Ser206Glu  
 + Ala209Gln + Leu211His + Asn212Gln  
 Asn198Gln + Gln200Glu + Pro204Gly + Gly205Gln + Ser206Asp  
 + Tyr208Met + Asn212Ser + Thr214Asn  
 Ala194Pro + Pro195Gln + Gly196Ser + Val197His + Gln200Glu  
 35 + Gly205Ser + Ser206Glu + Leu211Ser  
 Ala194Gln + Gln200Asp + Tyr203Gln + Pro204Asn + Ser206Glu  
 + Tyr208Ile + Ala209Gly + Gly213Asn  
 Ala194Gln + Pro195Gln + Val197His + Gln200Glu + Pro204Ser  
 + Ser206Asp + Tyr208Gln + Ala209Asn  
 40 Pro195Gln + Val197His + Val199Asn + Gln200Asp + Thr202Pro  
 + Ser206Glu + Leu211Thr + Thr214Pro  
 Pro195Gly + Asn198Gln + Val199Cys + Gln200Asp + Thr202Gly  
 + Ser206Glu + Tyr208Cys + Gly213Ser  
 Ala194Gly + Pro195Asn + Gly196Asn + Pro204Asp + Thr207Ser  
 45 + Leu211Glu + Asn212Gln + Thr214Asn

TABLE 31

## Loop 6 - Nonuple Mutation Variants

50 Ala194Gly + Pro195Ser + Val197Cys + Val199Asn + Gln200Asp  
 + Tyr203Pro + Pro204Ser + Gly205Ser + Leu211Gln  
 Ala194Pro + Gln200Asn + Thr202Asn + Tyr203Asn + Pro204Ser  
 + Tyr208Asn + Ala209Asn + Ser210Asp + Thr214Gln

Prol195Gly + Val199Ala + Gln200Ser + Thr202Gly + Gly205Pro  
 + Ser206Glu + Ala209His + Leu211Ala + Gly213Asn  
 Prol195Gln + Gly196Gln + Val197Thr + Val199His + Thr202Gln  
 + Tyr203Gly + Pro204Gly + Gly205Glu + Gly213Gln  
 5 Prol195Gly + Val197Gly + Val199Asn + Pro204Asn + Ser206Asp  
 + Thr207Ser + Tyr208Gln + Ala209Gly + Thr214Asn  
 Ala194Thr + Val197Asn + Val199Pro + Gln200Asp + Thr202Ser  
 + Tyr203Asn + Thr207Asn + Tyr208Leu + Ala209Gly  
 Prol195Asn + Gly196Asn + Asn198Gln + Tyr203Gln + Thr207Ser  
 10 + Ala209Thr + Leu211Ala + Asn212Asp + Thr214Asn  
 Ala194Gln + Prol195Gly + Gly196Pro + Val197Gly + Gln200Asp  
 + Thr202Ser + Pro204Gly + Tyr208Thr + Thr214Gln  
 Gly196Asn + Val199Gly + Tyr203Ser + Pro204Ser + Tyr208Gln  
 + Ala209His + Asn212Ser + Gly213Asp + Thr214Gln  
 15 Val197His + Gln200Ser + Thr202Asn + Pro204Ser + Thr207Pro  
 + Tyr208Gly + Ala209His + Ser210Glu + Leu211Ala  
 Prol195Ser + Val197His + Gln200Glu + Thr202Gln + Pro204Ser  
 + Tyr208Val + Ala209His + Leu211Thr + Thr214Pro  
 Prol195Gly + Gly196Pro + Val199Thr + Thr202Pro + Tyr203Ile  
 20 + Thr207Gly + Ala209Pro + Ser210Asp + Asn212Gln  
 Ala194Gly + Prol195Gln + Val197Asp + Tyr203Cys + Tyr208Val  
 + Ala209Gln + Leu211Gly + Asn212Gln + Gly213Gln  
 Gly196Asn + Asn198Gln + Thr202Gly + Thr207Asp + Tyr208Gly  
 + Ala209Ser + Leu211Thr + Asn212Ser + Thr214Pro  
 25 Ala194Pro + Gly196Asn + Val197Met + Gln200Glu + Tyr203Gln  
 + Gly205Asn + Tyr208Asn + Ala209Thr + Thr214Gln  
 Ala194Pro + Gly196Gln + Val197Asn + Gly205Ser + Ser206Asp  
 + Thr207Asn + Ala209Ser + Leu211Cys + Thr214Pro  
 Gly196Gln + Val197Thr + Gln200Ser + Tyr203Asn + Pro204Asn  
 30 + Gly205Pro + Tyr208Ser + Ser210Glu + Asn212Ser  
 Ala194Thr + Prol195Ser + Gly196Pro + Val197Cys + Gln200Glu  
 + Pro204Ser + Gly205Asn + Ala209His + Leu211Pro  
 Prol195Gln + Gly196Asn + Asn198Gln + Val199Gln + Gln200Asp  
 + Pro204Ser + Gly205Asn + Thr207Ser + Tyr208Ser  
 35 Ala194His + Val197Asn + Tyr203Gly + Pro204Gln + Thr207Gly  
 + Ala209Gln + Leu211His + Asn212Gln + Gly213Gln  
 Ala194Ser + Prol195Gln + Val197Met + Val199His + Thr207Pro  
 + Tyr208Ala + Asn212Gln + Gly213Glu + Thr214Asn  
 Gly196Pro + Val197His + Asn198Gln + Gln200Glu + Tyr203Ser  
 40 + Gly205Pro + Thr207Ser + Leu211Thr + Gly213Asn  
 Gly196Ser + Val197Asn + Val199Thr + Thr202Gly + Tyr203Asn  
 + Pro204Asn + Ala209Gln + Leu211Pro + Thr214Asp  
 Ala194Thr + Prol195Ser + Val197Asn + Tyr203Met + Tyr208Ala  
 + Ala209Ser + Asn212Gln + Gly213Asp + Thr214Asp  
 45 Ala194Asn + Gly196Gln + Val197Thr + Val199Ala + Gly205Asn  
 + Ala209Pro + Leu211Asn + Gly213Glu + Thr214Asp  
 Prol195Asn + Val199Met + Gln200Asn + Thr202Asn + Tyr203Cys  
 + Ala209Ser + Leu211Gln + Gly213Asp + Thr214Glu  
 Prol195Asn + Val197Met + Asn198Ser + Val199Asn + Gln200Asn  
 50 + Tyr208Gln + Leu211His + Gly213Glu + Thr214Asp  
 Val197His + Val199Ala + Gln200Ser + Pro204Ser + Ala209Glu  
 + Ser210Glu + Asn212Gln + Gly213Pro + Thr214Ser  
 Gly196Gln + Val197Asn + Asn198Gln + Thr202Gly + Gly205Gln  
 + Ala209Asp + Ser210Glu + Leu211Met + Thr214Gly

Vall197Cys + Asn198Ser + Vall199Gln + Tyr203Gly + Pro204Gln  
+ Ala209Glu + Ser210Glu + Gly213Pro + Thr214Gly  
Pro195Asn + Vall199His + Gln200Ser + Pro204Gln + Gly205Gln  
+ Ala209Glu + Ser210Asp + Leu211Gln + Asn212Gln  
5 Vall197Asp + Asn198Glu + Vall199Met + Thr202Gln + Pro204Ser  
+ Gly205Gln + Thr207Gln + Tyr208His + Asn212Ser  
Ala194Thr + Vall197Glu + Asn198Asp + Vall199His + Tyr203Asn  
+ Tyr208His + Ala209Ser + Gly213Ser + Thr214Asn  
Vall197Cys + Asn198Ser + Vall199Pro + Gln200Ser + Tyr203Ala  
10 + Tyr208Asn + Asn212Asp + Gly213Glu + Thr214Ser  
Gly196Asn + Vall197Met + Asn198Ser + Thr202Gly + Pro204Gln  
+ Ala209Ser + Leu211His + Asn212Asp + Gly213Asp  
Ala194Thr + Pro195Ser + Gln200Asn + Thr202Asn + Tyr203Gln  
+ Tyr208Ser + Leu211Ala + Asn212Asp + Gly213Asp  
15 Pro195Gly + Asn198Ser + Vall199Pro + Gly205Asp + Ser206Asp  
+ Thr207Pro + Tyr208Ala + Asn212Ser + Gly213Ser  
Pro195Gln + Asn198Ser + Gln200Asn + Thr202Ser + Tyr203Met  
+ Gly205Asp + Ser206Glu + Thr207Pro + Thr214Ser  
Gly196Ser + Vall199Thr + Gln200Asn + Tyr203Ala + Gly205Asp  
20 + Ser206Asp + Tyr208Leu + Ala209Thr + Gly213Pro  
Ala194Pro + Gly196Ser + Vall197Asn + Vall199His + Gln200Asn  
+ Gly205Glu + Ser206Glu + Asn212Gln + Thr214Gln  
Ala194Thr + Vall199Ser + Thr202Asn + Gly205Glu + Ser206Asp  
+ Ala209Pro + Leu211Pro + Asn212Gln + Gly213Gln  
25 Asn198Ser + Thr202Ser + Tyr203Ser + Pro204Gly + Gly205Asn  
+ Ser206Asp + Thr207Glu + Ala209Pro + Thr214Ser  
Pro195Asn + Gly196Asn + Vall197Pro + Vall199Ser + Thr202Ser  
+ Ser206Glu + Thr207Glu + Leu211Ile + Gly213Ser  
Thr202Ser + Tyr203Thr + Thr207Asp + Tyr208Glu + Ala209Pro  
30 + Leu211Val + Asn212Ser + Gly213Ser + Thr214Asn  
Vall199Pro + Gln200Ser + Thr202Ser + Thr207Asp + Tyr208Asp  
+ Ala209Gly + Leu211Met + Asn212Ser + Thr214Pro  
Ala194Gln + Pro195Gly + Vall197Ala + Gln200Glu + Tyr203Thr  
+ Gly205Ser + Ala209Pro + Ser210Asp + Thr214Asn  
35 Ala194Pro + Pro195Ser + Vall199Met + Gln200Glu + Thr202Gly  
+ Pro204Gln + Gly205Asn + Tyr208Gln + Ser210Asp  
Ala194Gln + Vall199Pro + Gln200Asp + Pro204Gly + Gly205Gln  
+ Thr207Pro + Tyr208Asn + Ala209Gln + Ser210Asp  
Ala194Pro + Gly196Pro + Asn198Gln + Gln200Asp + Pro204Gly  
40 + Gly205Asn + Tyr208Ile + Ser210Asp + Asn212Ser  
Pro195Ser + Asn198Ser + Vall199Ala + Gln200Glu + Tyr203Thr  
+ Gly205Gln + Tyr208Met + Ser210Glu + Gly213Pro  
Pro195Asn + Asn198Ser + Gln200Asp + Thr202Gln + Tyr203Ala  
+ Gly205Pro + Ser210Asp + Leu211Ser + Gly213Ser  
45 Pro195Asn + Gly196Asn + Vall197Ala + Asn198Ser + Gln200Asp  
+ Tyr203Gln + Pro204Asn + Ala209Ser + Ser210Asp  
Pro195Ser + Vall197Pro + Asn198Gln + Vall199Pro + Gln200Glu  
+ Thr202Gln + Thr207Asn + Ala209His + Ser210Glu  
Pro195Ser + Gly196Asn + Asn198Gln + Gln200Glu + Thr202Ser  
50 + Pro204Gly + Thr207Asn + Ser210Glu + Thr214Asn  
Ala194Ser + Pro195Ser + Vall197His + Vall199Thr + Gln200Glu  
+ Gly205Gln + Thr207Gly + Ser210Glu + Gly213Pro  
Pro195Asn + Vall197Asp + Asn198Ser + Vall199Asn + Thr207Pro  
+ Tyr208Ala + Leu211Val + Asn212Glu + Gly213Ser



Pro195Asn + Gly196Asn + Val197Gln + Asn198Glu + Val199Thr  
+ Tyr203Asn + Thr207Pro + Ser210Glu + Gly213Gln  
Val197Cys + Asn198Asp + Val199Ser + Tyr203Gln + Gly205Ser  
+ Thr207Ser + Tyr208Leu + Ser210Asp + Leu211Gly  
5 Ala194Pro + Gly196Gln + Val197Gly + Asn198Glu + Gln200Ser  
+ Tyr203Thr + Pro204Ser + Ala209His + Ser210Glu  
Gly196Ser + Val197His + Asn198Glu + Thr202Pro + Tyr203Ile  
+ Thr207Asn + Tyr208Val + Ser210Glu + Thr214Ser  
Gly196Asn + Val197Thr + Asn198Asp + Val199Ala + Pro204Asn  
10 + Ser210Asp + Leu211Val + Gly213Gln + Thr214Pro  
Gly196Ser + Asn198Glu + Thr202Asn + Gly205Ser + Thr207Gln  
+ Tyr208Ile + Ser210Glu + Leu211Ser + Asn212Ser  
Ala194Asn + Gly196Pro + Asn198Asp + Thr202Gly + Tyr208Ser  
+ Ala209Pro + Ser210Glu + Leu211Ala + Thr214Asn  
15 Ala194His + Asn198Glu + Val199Gly + Gln200Asn + Gly205Asn  
+ Ser210Glu + Leu211His + Asn212Gln + Thr214Ser  
Ala194Gly + Asn198Glu + Gln200Asn + Thr202Pro + Tyr203Cys  
+ Thr207Gln + Tyr208Met + Ser210Asp + Asn212Gln  
Pro195Gly + Gly196Gln + Asn198Glu + Gln200Ser + Pro204Gln  
20 + Tyr208Leu + Ala209Thr + Ser210Glu + Leu211Ile  
Ala194Pro + Pro195Gln + Val197His + Asn198Glu + Thr202Gln  
+ Gly205Pro + Thr207Asn + Tyr208Val + Leu211Asp  
Pro195Gln + Asn198Glu + Thr202Ser + Tyr203Gly + Pro204Ser  
+ Thr207Gln + Ala209Pro + Leu211Asp + Gly213Asn  
25 Val199Asn + Gln200Asp + Thr202Gln + Tyr203Gly + Pro204Asn  
+ Thr207Gln + Tyr208Ala + Ala209Glu + Asn212Ser  
Gly196Pro + Val197Thr + Pro204Asp + Ser206Glu + Ala209Gln  
+ Leu211Gln + Asn212Ser + Gly213Ser + Thr214Pro  
Gly196Pro + Asn198Ser + Val199Thr + Pro204Glu + Ser206Asp  
30 + Thr207Gln + Ala209Gly + Asn212Ser + Thr214Gly  
Ala194Asn + Val197Gly + Gln200Ser + Thr202Ser + Pro204Asp  
+ Ser206Asp + Tyr208Gln + Ala209Gln + Leu211Gly  
Ala194Pro + Thr202Gln + Tyr203Asn + Pro204Asp + Ser206Glu  
+ Tyr208Gly + Ala209Ser + Gly213Pro + Thr214Pro  
35 Ala194Asn + Pro195Gln + Val197Thr + Asn198Ser + Pro204Asp  
+ Gly205Ser + Ser206Asp + Asn212Ser + Thr214Asn  
Pro195Gly + Gln200Ser + Thr202Gln + Tyr203Pro + Pro204Asp  
+ Gly205Asn + Ser206Glu + Thr207Asn + Leu211His  
Ala194His + Gly196Asn + Val197Met + Asn198Ser + Pro204Asp  
40 + Ser206Asp + Leu211Asn + Asn212Ser + Thr214Gln  
Asn198Gln + Val199Cys + Gln200Ser + Thr202Gln + Pro204Asp  
+ Gly205Gln + Ser206Asp + Tyr208Leu + Thr214Pro  
Ala194His + Pro195Ser + Gly196Pro + Asn198Gln + Pro204Glu  
+ Ser206Asp + Thr207Pro + Tyr208His + Ala209Asn  
45 Ala194Ser + Asn198Asp + Gln200Ser + Thr202Gly + Thr207Pro  
+ Tyr208Asn + Ala209Gln + Leu211Ser + Asn212Glu  
Gly196Asn + Asn198Gln + Gln200Asn + Tyr203Thr + Pro204Ser  
+ Gly205Asp + Thr207Glu + Ala209Ser + Thr214Asn  
Val197His + Asn198Gln + Val199Met + Gln200Ser + Pro204Glu  
50 + Gly205Ser + Thr207Glu + Tyr208Val + Thr214Gly  
Val197Asn + Asn198Asp + Val199Ala + Gln200Glu + Tyr203Cys  
+ Pro204Gly + Gly205Asn + Thr207Gln + Gly213Asn  
Pro195Asn + Gly196Gln + Asn198Asp + Val199Pro + Gln200Glu  
+ Tyr203Val + Gly205Asn + Tyr208Cys + Ala209Pro

Pro195Gly + Gly196Pro + Val197Asp + Thr202Ser + Pro204Gln  
+ Thr207Gly + Tyr208Ile + Asn212Gln + Gly213Glu  
Ala194Pro + Val197Glu + Tyr203His + Gly205Pro + Thr207Gly  
+ Ala209Asn + Leu211His + Gly213Asp + Thr214Gly  
5 Pro195Gln + Val197Glu + Asn198Gln + Val199Ala + Pro204Ser  
+ Gly205Gln + Ala209Ser + Gly213Glu + Thr214Ser  
Pro195Asn + Val197Asp + Asn198Gln + Val199Ser + Gln200Ser  
+ Thr207Ser + Tyr208Ile + Asn212Ser + Gly213Asp  
Gly196Pro + Val197Glu + Asn198Gln + Val199Asn + Pro204Gly  
10 + Thr207Pro + Leu211His + Gly213Asp + Thr214Asn  
Ala194Ser + Asn198Gln + Thr202Pro + Tyr203Met + Pro204Ser  
+ Thr207Gln + Ala209Asp + Leu211Glu + Thr214Gln  
Gly196Pro + Val197Pro + Thr202Asn + Tyr203Cys + Tyr208Gln  
+ Ser210Asp + Asn212Asp + Gly213Pro + Thr214Ser  
15 Pro195Gln + Tyr203Thr + Gly205Gln + Ala209Gln + Ser210Asp  
+ Leu211Asn + Asn212Glu + Gly213Asn + Thr214Gln  
Ala194Gly + Pro195Ser + Val197His + Thr202Gln + Pro204Gly  
+ Ser210Asp + Leu211His + Asn212Glu + Thr214Gly  
Pro195Asn + Gly196Asn + Val197Asn + Thr202Ser + Tyr203Val  
20 + Gly205Ser + Ser210Asp + Asn212Glu + Gly213Pro  
Gln200Ser + Thr202Gly + Tyr203Leu + Thr207Pro + Tyr208Pro  
+ Ser210Asp + Leu211Pro + Asn212Glu + Thr214Gly  
Ala194Gln + Gly196Ser + Val197Cys + Val199Met + Tyr203Leu  
+ Ala209Thr + Ser210Asp + Asn212Glu + Thr214Ser  
25 Val197Met + Val199Ala + Gln200Asn + Tyr203Thr + Thr207Gln  
+ Tyr208Glu + Ser210Glu + Asn212Ser + Thr214Gly  
Gly196Ser + Val197Ala + Val199Ser + Thr202Gly + Tyr203Cys  
+ Gly205Asn + Ser206Glu + Tyr208Asp + Leu211Ile  
Ala194Gly + Pro195Ser + Val199His + Thr202Asn + Gly205Gln  
30 + Ser206Asp + Tyr208Glu + Asn212Ser + Thr214Pro  
Gly196Pro + Val199Gln + Thr202Ser + Gly205Pro + Ser206Asp  
+ Thr207Gln + Tyr208Glu + Leu211His + Asn212Ser  
Val197His + Asn198Ser + Gly205Ser + Thr207Pro + Tyr208Val  
+ Leu211Val + Asn212Glu + Gly213Ser + Thr214Glu  
35 Pro195Ser + Gly196Pro + Val199Thr + Tyr203Ser + Tyr208Gly  
+ Ala209Asn + Asn212Glu + Gly213Ser + Thr214Glu  
Pro195Asn + Asn198Gln + Gln200Asn + Thr202Asn + Thr207Asn  
+ Tyr208Gln + Asn212Glu + Gly213Ser + Thr214Glu  
Ala194Asn + Val199Ser + Thr202Gly + Pro204Asn + Gly205Ser  
40 + Ala209Asn + Leu211Asn + Asn212Asp + Thr214Glu  
Ala194Ser + Gly196Gln + Pro204Asp + Gly205Pro + Thr207Pro  
+ Tyr208Glu + Leu211Ile + Gly213Asn + Thr214Ser  
Pro195Ser + Val197Asp + Asn198Gln + Gln200Asn + Tyr203Pro  
+ Gly205Asn + Ser210Asp + Asn212Ser + Thr214Asn  
45 Ala194His + Gly196Ser + Val197Asp + Asn198Ser + Gln200Asn  
+ Thr202Ser + Gly205Gln + Ser210Glu + Asn212Ser  
Ala194Thr + Val197Glu + Thr202Gln + Pro204Gln + Tyr208Gly  
+ Ala209Thr + Ser210Glu + Asn212Ser + Thr214Gln  
Gly196Ser + Val197Asp + Val199Pro + Tyr203Cys + Pro204Ser  
50 + Thr207Asn + Tyr208Asn + Ser210Asp + Asn212Ser  
Ala194Pro + Pro195Ser + Gly196Gln + Val197Asp + Val199Met  
+ Gln200Asn + Leu211Met + Gly213Pro + Thr214Asp  
Pro195Asn + Gly196Gln + Asn198Gln + Val199Ala + Gln200Ser  
+ Tyr203Pro + Gly205Glu + Thr207Asn + Tyr208Asp

Gly196Pro + Asn198Ser + Val199Asn + Thr202Gly + Pro204Gln  
+ Gly205Asp + Thr207Gln + Tyr208Asp + Thr214Asn  
Val197Ser + Asn198Glu + Gln200Asn + Tyr203Gln + Pro204Asn  
+ Gly205Pro + Thr207Gly + Ala209Thr + Gly213Glu  
5 Ala194Thr + Gly196Gln + Val197His + Asn198Asp + Val199Ser  
+ Tyr203Pro + Tyr208Cys + Gly213Asp + Thr214Pro  
Asn198Asp + Gln200Ser + Tyr203Gln + Pro204Ser + Gly205Asn  
+ Thr207Asn + Ala209Glu + Leu211Gln + Asn212Ser  
Pro195Gly + Gly196Asn + Asn198Glu + Thr202Gln + Tyr203Ala  
10 + Pro204Ser + Thr207Pro + Ala209Glu + Gly213Gln  
Ala194Pro + Asn198Glu + Val199Thr + Pro204Gly + Thr207Gly  
+ Tyr208Ile + Ala209Asp + Leu211Met + Asn212Ser  
Ala194Ser + Val197Glu + Val199Thr + Gln200Asp + Tyr203Gly  
+ Gly205Ser + Tyr208His + Ala209Thr + Asn212Ser  
15 Gly196Gln + Val197Glu + Val199Pro + Gln200Asp + Gly205Gln  
+ Thr207Ser + Ala209Thr + Leu211Pro + Thr214Gly  
Val197Gly + Gln200Glu + Thr202Gly + Tyr203Leu + Gly205Gln  
+ Thr207Gly + Tyr208Leu + Ala209Asn + Asn212Asp  
Pro195Ser + Gly196Asn + Asn198Ser + Val199His + Gln200Glu  
20 + Tyr203Pro + Ala209Ser + Asn212Asp + Thr214Asn  
Asn198Ser + Val199Met + Gln200Glu + Thr202Ser + Tyr203Ile  
+ Gly205Pro + Ala209Pro + Asn212Glu + Gly213Gln  
Gly196Pro + Val197Ser + Val199Pro + Gln200Glu + Ala209Gly  
+ Leu211Gln + Asn212Asp + Gly213Pro + Thr214Asn  
25 Ala194Gln + Gly196Ser + Val197Pro + Thr202Pro + Pro204Asp  
+ Tyr208Gly + Ala209Glu + Gly213Asn + Thr214Pro  
Ala194Pro + Val197Ser + Val199His + Gln200Asn + Pro204Gly  
+ Ser206Glu + Thr207Asn + Tyr208Leu + Ala209Glu  
Gly196Asn + Thr202Asn + Pro204Asn + Gly205Pro + Ser206Glu  
30 + Tyr208Asn + Ala209Asp + Leu211Met + Thr214Gln  
Pro195Gly + Asn198Gln + Val199Ser + Gln200Ser + Tyr203Thr  
+ Gly205Pro + Ser206Glu + Ala209Glu + Thr214Asn  
Ala194Thr + Val197Ala + Thr202Ser + Tyr203Pro + Gly205Asn  
+ Ser206Glu + Ala209Glu + Leu211Gln + Thr214Asn  
35 Pro195Ser + Val197Ala + Thr202Asn + Pro204Gly + Ser206Asp  
+ Thr207Asn + Ala209Glu + Asn212Ser + Thr214Ser  
Ala194Thr + Val199Asn + Thr202Pro + Tyr203Ile + Pro204Asn  
+ Ser206Asp + Thr207Asn + Ala209Glu + Thr214Asn  
Ala194Ser + Pro195Asn + Val197Gly + Val199Gly + Gln200Glu  
40 + Thr202Gln + Pro204Asn + Thr207Asp + Leu211Gln  
Ala194Gly + Val197His + Gln200Ser + Pro204Asn + Thr207Ser  
+ Ser210Glu + Leu211Gly + Gly213Glu + Thr214Asn  
Ala194Gln + Gly196Gln + Val197Met + Val199His + Thr202Pro  
+ Gly205Asn + Ala209Ser + Ser210Glu + Gly213Glu  
45 Pro195Asn + Val197Thr + Val199Ala + Gln200Asn + Tyr203Asn  
+ Thr207Gly + Ser210Asp + Asn212Gln + Gly213Glu  
Ala194His + Val199Pro + Gln200Asn + Thr202Pro + Tyr203Asn  
+ Gly205Glu + Ala209Glu + Asn212Gln + Thr214Asn  
Gly196Pro + Asn198Gln + Gln200Asn + Tyr203Gln + Pro204Gln  
50 + Gly205Asp + Ala209Glu + Asn212Gln + Thr214Gln  
Pro195Gln + Asn198Gln + Val199Ala + Gln200Asn + Thr207Glu  
+ Tyr208Leu + Ala209Ser + Ser210Asp + Gly213Pro  
Pro195Gly + Gly196Gln + Val199Ser + Gln200Asn + Pro204Ser  
+ Gly205Ser + Thr207Glu + Ser210Glu + Thr214Pro

Gly196Gln + Gln200Ser + Thr202Gly + Tyr203Ile + Thr207Asp  
+ Tyr208Ile + Ser210Asp + Leu211Asn + Asn212Ser  
Ala194His + Gly196Gln + Val197Gly + Thr207Glu + Tyr208Val  
+ Ala209Gln + Ser210Asp + Leu211His + Gly213Ser  
5 Ala194Gln + Asn198Asp + Val199Ser + Gln200Ser + Tyr203Ile  
+ Gly205Asn + Thr207Asn + Asn212Gln + Thr214Asp  
Val197Pro + Asn198Glu + Val199Thr + Gln200Ser + Pro204Asn  
+ Gly205Pro + Leu211Cys + Asn212Ser + Thr214Asp  
Ala194Ser + Val199Thr + Gln200Glu + Tyr203Cys + Tyr208Gly  
10 + Ala209His + Leu211Gly + Asn212Ser + Gly213Glu  
Gly196Gln + Asn198Gln + Val199Met + Gln200Asp + Pro204Gln  
+ Tyr208Pro + Asn212Gln + Gly213Asp + Thr214Gln  
Val197Asp + Val199Gln + Gln200Ser + Thr202Pro + Thr207Gln  
+ Ala209Glu + Leu211Gly + Asn212Ser + Gly213Gln  
15 Ala194Ser + Gly196Pro + Val197Glu + Asn198Ser + Val199Met  
+ Pro204Gly + Thr207Gln + Ala209Glu + Gly213Gln  
Val197Asp + Asn198Ser + Pro204Gln + Gly205Gln + Thr207Gly  
+ Tyr208Met + Ala209Glu + Asn212Gln + Thr214Gln  
Ala194Ser + Val197Glu + Asn198Gln + Tyr203Asn + Gly205Asn  
20 + Ala209Glu + Asn212Gln + Gly213Ser + Thr214Pro  
Pro195Gly + Val197Ser + Asn198Ser + Tyr203Leu + Pro204Glu  
+ Ser210Asp + Leu211Ser + Asn212Ser + Thr214Gln  
Ala194Asn + Pro195Gln + Asn198Gln + Val199Asn + Gln200Asn  
+ Pro204Asp + Thr207Pro + Ser210Glu + Gly213Gln  
25 Ala194Pro + Val197Ser + Asn198Ser + Thr202Pro + Tyr203Val  
+ Pro204Glu + Ser210Asp + Asn212Ser + Thr214Asn  
Ala194Gln + Pro195Ser + Gly196Pro + Val199Ser + Pro204Asp  
+ Tyr208Cys + Ala209Thr + Ser210Glu + Gly213Asn  
Pro195Ser + Tyr203Pro + Pro204Asp + Gly205Pro + Thr207Gln  
30 + Ala209Gly + Ser210Asp + Leu211Asn + Thr214Asn  
Gly196Ser + Asn198Ser + Thr202Pro + Pro204Asp + Tyr208Val  
+ Ala209Gln + Ser210Asp + Leu211Asn + Asn212Gln  
Gly196Ser + Val199Met + Tyr203Ala + Pro204Glu + Gly205Ser  
+ Thr207Ser + Tyr208Leu + Ala209Gln + Ser210Asp  
35 Pro195Gly + Val197Pro + Thr202Asn + Pro204Glu + Gly205Ser  
+ Thr207Asn + Tyr208Pro + Ser210Glu + Asn212Ser  
Ala194Asn + Gly196Ser + Asn198Ser + Val199Met + Gln200Asn  
+ Thr202Gly + Thr207Gln + Ala209Asp + Gly213Asp  
Ala194Gln + Pro195Gln + Thr202Ser + Pro204Asn + Ala209Thr  
40 + Ser210Glu + Leu211Asn + Gly213Gln + Thr214Glu  
Gly196Gln + Val197Pro + Asn198Gln + Val199Gly + Ala209Pro  
+ Ser210Asp + Leu211Cys + Asn212Ser + Thr214Glu  
Ala194Asn + Pro195Gly + Asn198Gln + Val199His + Tyr203Pro  
+ Thr207Gly + Tyr208Met + Ser210Asp + Thr214Asp  
45 Ala194Pro + Pro195Ser + Gly196Asn + Thr202Gly + Pro204Ser  
+ Ala209His + Ser210Glu + Leu211Ser + Thr214Asp  
Ala194Thr + Gly196Pro + Asn198Ser + Thr207Asn + Tyr208Ile  
+ Ser210Asp + Leu211Val + Gly213Gln + Thr214Glu  
Ala194Gly + Pro195Ser + Asn198Ser + Thr202Ser + Gly205Ser  
50 + Ala209Gln + Ser210Asp + Asn212Ser + Thr214Asp  
Ala194Ser + Pro195Gly + Gly196Ser + Val199Ala + Tyr203Gln  
+ Pro204Gln + Thr207Ser + Ser210Glu + Thr214Glu  
Gly196Ser + Gln200Asn + Thr202Pro + Tyr203Leu + Thr207Gln  
+ Ser210Asp + Leu211Ala + Gly213Pro + Thr214Asp

Ala194Gln + Asn198Ser + Val199Thr + Gln200Ser + Tyr203Thr  
 + Ala209Pro + Ser210Asp + Leu211Asn + Thr214Glu  
 Pro195Gln + Gly196Gln + Tyr203Cys + Pro204Ser + Gly205Pro  
 + Tyr208His + Ala209Thr + Ser210Asp + Thr214Glu  
 5 Gly196Gln + Asn198Ser + Val199Thr + Gln200Ser + Thr202Ser  
 + Thr207Ser + Ala209His + Ser210Glu + Thr214Glu  
 Pro195Asn + Gly196Pro + Asn198Gln + Tyr203Ala + Thr207Ser  
 + Ala209Pro + Ser210Asp + Leu211Thr + Thr214Asp  
 Ala194Ser + Pro195Asn + Val199Pro + Pro204Asn + Gly205Gln  
 10 + Thr207Gln + Ser210Glu + Gly213Ser + Thr214Asp  
 Gly196Ser + Val197Asn + Thr202Gln + Tyr203Ser + Pro204Ser  
 + Gly205Ser + Ser210Glu + Leu211Cys + Thr214Asp  
 Val197Asn + Gln200Asp + Tyr203Cys + Pro204Asn + Gly205Asn  
 + Ser206Asp + Thr207Ser + Tyr208Leu + Asn212Gln  
 15 Pro195Asn + Gln200Asp + Thr202Gln + Tyr203Gln + Pro204Ser  
 + Ser206Glu + Thr207Pro + Asn212Ser + Gly213Gln  
 Ala194Gln + Pro195Gln + Gly196Asn + Val199Ser + Gln200Asp  
 + Thr202Ser + Gly205Ser + Ser206Glu + Ala209Ser  
 Pro195Asn + Gly196Pro + Val197Cys + Val199Met + Gln200Asp  
 20 + Ser206Glu + Thr207Ser + Ala209Gln + Leu211Val  
 Pro195Gly + Val199Pro + Gln200Asp + Thr202Ser + Tyr203Gly  
 + Pro204Gln + Ser206Asp + Leu211Met + Asn212Ser  
 Gly196Pro + Val197Cys + Asn198Gln + Gln200Glu + Thr202Asn  
 + Ser206Asp + Ala209Asn + Leu211Cys + Gly213Ser  
 25 Pro195Gln + Asn198Ser + Gln200Glu + Thr202Asn + Pro204Gly  
 + Ser206Asp + Thr207Ser + Tyr208Ser + Asn212Gln  
 Gly196Asn + Asn198Ser + Val199Met + Gln200Asp + Thr202Ser  
 + Tyr203His + Pro204Asn + Gly205Gln + Ser206Glu  
 Ala194Thr + Asn198Gln + Val199Ala + Gln200Asp + Thr202Asn  
 30 + Ser206Glu + Thr207Ser + Tyr208His + Gly213Gln  
 Ala194Gln + Gly196Gln + Asn198Gln + Gln200Asp + Thr202Asn  
 + Ser206Glu + Thr207Ser + Tyr208Thr + Gly213Gln

TABLE 32

	Loop 6 - Decuple Mutation Variants
35	Ala194Ser + Gly196Gln + Val197Ala + Asn198Gln + Thr202Pro + Pro204Ser + Gly205Ser + Thr207Pro + Leu211Val + Asn212Asp
40	Pro195Ser + Val197Gln + Val199His + Thr202Asn + Tyr203Pro + Gly205Gln + Ala209Thr + Ser210Glu + Asn212Ser + Thr214Gln
	Ala194Gly + Asn198Ser + Val199Ala + Thr202Gln + Tyr203Leu + Pro204Gly + Gly205Pro + Ala209His + Gly213Ser + Thr214Asp
45	Ala194His + Gly196Gln + Thr202Gly + Tyr203Val + Gly205Asn + Thr207Gln + Tyr208Cys + Ala209Ser + Gly213Gln + Thr214Asn
	Ala194Thr + Pro195Ser + Gly196Asn + Asn198Glu + Gln200Asn + Thr202Gly + Pro204Ser + Asn212Gln + Gly213Pro + Thr214Gly
50	Ala194Ser + Pro195Asn + Gly196Asn + Val199Thr + Thr202Gln + Pro204Asp + Thr207Asn + Tyr208Ser + Leu211Asn +

- Asn212Gln  
 Pro195Asn + Gly196Ser + Val197Gly + Asn198Ser + Thr202Gly  
 + Gly205Pro + Thr207Pro + Tyr208Asp + Ala209Ser +  
 Thr214Asn
- 5   Ala194His + Pro195Gln + Gly196Asn + Asn198Gln + Val199His  
 + Pro204Ser + Gly205Asn + Thr207Ser + Tyr208Ser +  
 Thr214Ser  
 Ala194Pro + Pro195Asn + Val197Asn + Val199Cys + Gln200Ser  
 + Thr202Pro + Pro204Ser + Ser206Glu + Tyr208His +
- 10   Leu211Ile  
 Ala194Thr + Val197Met + Asn198Asp + Val199Gly + Gln200Ser  
 + Tyr203Gln + Pro204Asn + Thr207Gln + Ala209Pro +  
 Leu211Ala  
 Ala194Pro + Pro195Gly + Val197Asn + Asn198Ser + Val199His  
 + Tyr208His + Ala209Asp + Leu211Thr + Asn212Gln +
- 15   Thr214Gln  
 Pro195Ser + Val199Met + Thr202Ser + Tyr203Ile + Gly205Gln  
 + Ser206Asp + Tyr208His + Leu211Pro + Asn212Gln +  
 Gly213Pro
- 20   Ala194Thr + Val197Ser + Val199Asn + Thr202Gln + Tyr203Met  
 + Gly205Pro + Ser206Glu + Tyr208Ile + Ala209Asn +  
 Gly213Ser  
 Ala194Thr + Val197Gln + Asn198Gln + Val199Gly + Thr202Ser  
 + Pro204Ser + Gly205Pro + Thr207Asn + Ala209Pro +
- 25   Thr214Asn  
 Ala194His + Pro195Asn + Gly196Asn + Val199Thr + Gln200Asn  
 + Thr202Asn + Tyr203Ala + Gly205Asp + Thr207Gly +  
 Thr214Pro  
 Ala194Ser + Pro195Gly + Gly196Ser + Val197Pro + Asn198Gln  
 + Val199Thr + Thr202Pro + Tyr203Met + Pro204Gln +
- 30   Gly205Gln  
 Ala194Thr + Pro195Gln + Val197Asp + Gln200Ser + Thr202Pro  
 + Pro204Ser + Gly205Gln + Thr207Gly + Tyr208His +  
 Ala209Gly
- 35   Ala194Pro + Pro195Ser + Asn198Ser + Val199Gln + Gln200Asp  
 + Thr202Gln + Tyr203Asn + Pro204Gly + Leu211Val +  
 Thr214Pro  
 Pro195Ser + Gly196Gln + Val199Pro + Gln200Asn + Tyr203Gln  
 + Thr207Asp + Ala209His + Leu211His + Asn212Gln +
- 40   Thr214Gly  
 Gly196Pro + Asn198Ser + Val199Gly + Gln200Ser + Thr202Asn  
 + Tyr203Ser + Tyr208Leu + Ala209His + Leu211Asp +  
 Asn212Asp  
 Gly196Asn + Val199Ala + Gln200Ser + Thr202Gln + Tyr203His  
 + Pro204Asn + Gly205Pro + Asn212Gln + Gly213Glu +
- 45   Thr214Glu  
 Gly196Pro + Val199Ser + Gln200Ser + Thr202Pro + Pro204Gln  
 + Ala209Glu + Ser210Glu + Asn212Ser + Gly213Ser +  
 Thr214Ser
- 50   Val197Thr + Asn198Gln + Val199Gln + Pro204Gly + Thr207Pro  
 + Ala209Asp + Ser210Glu + Asn212Ser + Gly213Ser +  
 Thr214Ser  
 Ala194Pro + Pro195Gln + Val197Ala + Val199Gln + Gln200Ser  
 + Pro204Gln + Gly205Pro + Tyr208Met + Ala209Asp +

- Ser210Glu  
Pro195Gly + Val197Gly + Asn198Gln + Thr202Gly + Tyr203Ala  
+ Gly205Pro + Ala209Glu + Ser210Glu + Leu211Ser +  
Gly213Gln
- 5 Pro195Ser + Gly196Asn + Asn198Gln + Gln200Ser + Thr202Gly  
+ Pro204Gln + Tyr208Thr + Leu211Cys + Asn212Glu +  
Gly213Glu  
Gly196Gln + Val197Ser + Gln200Ser + Thr202Pro + Pro204Asp  
+ Gly205Asp + Tyr208Gln + Ala209Pro + Asn212Ser +
- 10 Thr214Gln  
Ala194Pro + Pro195Gln + Gly196Gln + Pro204Asp + Gly205Glu  
+ Tyr208Ala + Ala209Ser + Leu211Asn + Gly213Asn +  
Thr214Ser
- 15 Ala194Thr + Val197Cys + Val199Met + Gln200Ser + Gly205Pro  
+ Tyr208Thr + Ser210Glu + Leu211Asp + Asn212Ser +  
Gly213Pro  
Ala194Gly + Val197His + Thr202Ser + Tyr203Asn + Thr207Pro  
+ Tyr208Cys + Ser210Glu + Leu211Glu + Asn212Gln +  
Gly213Pro
- 20 Val197His + Asn198Ser + Gln200Ser + Thr202Asn + Gly205Ser  
+ Tyr208Gln + Ser210Glu + Leu211Glu + Asn212Ser +  
Thr214Pro  
Ala194Thr + Gly196Asn + Asn198Gln + Gln200Asn + Thr202Pro  
+ Tyr203Thr + Gly205Asn + Ser210Glu + Leu211Glu +  
Asn212Ser
- 25 Ala194Gly + Gly196Gln + Asn198Ser + Val199Cys + Pro204Asn  
+ Gly205Asp + Ser206Asp + Thr207Gln + Ala209Gly +  
Gly213Asn  
Pro195Gln + Val197Met + Val199Ala + Gln200Ser + Gly205Asp  
+ Ser206Asp + Thr207Asn + Tyr208Gln + Leu211Pro +  
Asn212Ser
- 30 Ala194His + Pro195Gln + Asn198Gln + Thr202Pro + Gly205Glu  
+ Ser206Asp + Thr207Pro + Leu211Thr + Gly213Ser +  
Thr214Gln
- 35 Gly196Pro + Asn198Gln + Val199Gly + Gln200Ser + Thr202Asn  
+ Pro204Asn + Gly205Glu + Ser206Asp + Thr207Gly +  
Thr214Gln  
Pro195Gln + Val197His + Asn198Gln + Thr202Gly + Gly205Asp  
+ Ser206Glu + Thr207Ser + Tyr208Ala + Asn212Gln +  
Gly213Asn
- 40 Ala194Asn + Gly196Asn + Val197Ser + Asn198Ser + Val199Met  
+ Thr202Asn + Tyr203Ala + Gly205Asp + Ser206Asp +  
Thr214Asn  
Ala194Thr + Pro195Asn + Gly196Gln + Val199Thr + Gln200Asn  
+ Thr202Ser + Pro204Asn + Gly205Asp + Ser206Glu +  
Thr207Asn
- 45 Pro195Ser + Gly196Ser + Val197Pro + Asn198Gln + Gln200Asn  
+ Tyr203Leu + Gly205Asp + Ser206Glu + Asn212Ser +  
Thr214Pro
- 50 Pro195Ser + Gly196Pro + Val197Ser + Gln200Ser + Thr202Pro  
+ Tyr203Val + Pro204Gly + Gly205Glu + Ser206Glu +  
Tyr208Met  
Ala194Gly + Gly196Gln + Gln200Ser + Thr202Ser + Pro204Asn  
+ Ser206Asp + Thr207Asp + Ala209Gln + Leu211Met +

Thr214Pro  
 Gly196Asn + Val197Ser + Val199Ala + Tyr203Ile + Ser206Asp  
 + Thr207Glu + Ala209Ser + Leu211Ala + Asn212Ser +  
 Thr214Asn  
 5 Ala194Ser + Pro204Asn + Gly205Ser + Ser206Asp + Thr207Glu  
 + Tyr208Met + Ala209Asn + Leu211Ile + Asn212Gln +  
 Gly213Asn  
 Ala194Gln + Asn198Gln + Val199Asn + Thr202Gln + Tyr203Ile  
 + Gly205Gln + Ser206Asp + Thr207Glu + Tyr208Ser +  
 10 Gly213Ser  
 Gly196Gln + Asn198Gln + Thr202Gln + Pro204Ser + Gly205Ser  
 + Ser206Glu + Thr207Asp + Leu211Ile + Gly213Pro +  
 Thr214Pro  
 Pro195Asn + Gly196Gln + Val197Gln + Thr202Asn + Gly205Ser  
 15 + Ser206Glu + Thr207Glu + Tyr208Ile + Gly213Gln +  
 Thr214Asn  
 Asn198Ser + Thr202Ser + Tyr203Ile + Gly205Asn + Thr207Asn  
 + Tyr208Asp + Ala209Glu + Leu211Ile + Gly213Ser +  
 Thr214Ser  
 20 Val199His + Gln200Glu + Tyr203Met + Pro204Gly + Thr207Pro  
 + Ala209Thr + Ser210Glu + Leu211Asn + Asn212Ser +  
 Gly213Pro  
 Ala194His + Gly196Ser + Asn198Ser + Gln200Glu + Thr202Gln  
 + Tyr203Gln + Gly205Pro + Thr207Gly + Ser210Glu +  
 25 Gly213Asn  
 Gly196Pro + Val199Asn + Gln200Glu + Pro204Gly + Tyr208His  
 + Ser210Asp + Leu211Gln + Asn212Ser + Gly213Gln +  
 Thr214Asn  
 Pro195Gly + Gly196Pro + Gln200Asp + Tyr203Asn + Pro204Asn  
 30 + Gly205Asn + Thr207Asn + Tyr208Ile + Ser210Asp +  
 Gly213Gln  
 Ala194Gln + Pro195Gln + Gly196Gln + Val197Gln + Gln200Asp  
 + Thr207Gly + Ala209Asn + Ser210Asp + Leu211Gly +  
 Gly213Asn  
 35 Val197Thr + Val199Ser + Gln200Glu + Tyr203Asn + Pro204Gly  
 + Thr207Asn + Tyr208Gly + Ser210Glu + Asn212Ser +  
 Thr214Asn  
 Gly196Gln + Val197Gln + Val199Asn + Gln200Glu + Tyr203Asn  
 + Thr207Gln + Ala209Gly + Ser210Glu + Asn212Gln +  
 40 Gly213Gln  
 Pro195Asn + Val197Met + Val199Cys + Gln200Asp + Thr202Asn  
 + Thr207Ser + Tyr208Gly + Ala209Gln + Ser210Glu +  
 Leu211Asn  
 Ala194Asn + Val199Gln + Gln200Asp + Tyr203Gly + Pro204Ser  
 45 + Gly205Pro + Tyr208Ile + Ser210Glu + Leu211Asn +  
 Gly213Ser  
 Ala194His + Pro195Gln + Val197Glu + Asn198Ser + Val199Ser  
 + Tyr203His + Thr207Pro + Leu211Gln + Asn212Glu +  
 Gly213Ser  
 50 Gly196Ser + Val197Glu + Asn198Gln + Val199Cys + Tyr203Pro  
 + Pro204Gly + Ala209Gln + Asn212Glu + Gly213Gln +  
 Thr214Asn  
 Gly196Ser + Val197Gly + Asn198Asp + Gln200Ser + Tyr203Pro  
 + Tyr208Ala + Ala209Gly + Ser210Glu + Leu211Ile +



- Thr214Asn  
 Gly196Pro + Asn198Asp + Val199Gln + Gln200Asn + Pro204Gln  
 + Gly205Pro + Thr207Gly + Ser210Asp + Asn212Gln +  
 Gly213Ser
- 5 Pro195Asn + Gly196Ser + Val197Gln + Asn198Glu + Gln200Ser  
 + Pro204Gly + Tyr208Gln + Ala209Asn + Ser210Glu +  
 Gly213Pro
- Asn198Asp + Val199Met + Gln200Asn + Thr202Gln + Pro204Asn  
 + Gly205Gln + Tyr208Ala + Ala209His + Ser210Glu +
- 10 Thr214Pro  
 Ala194Gln + Val197Ala + Asn198Glu + Gln200Asn + Thr202Gly  
 + Pro204Gln + Gly205Gln + Tyr208Thr + Ser210Asp +  
 Leu211Val
- Val197Ser + Asn198Asp + Val199Asn + Gln200Ser + Pro204Ser  
 + Thr207Pro + Tyr208Leu + Ser210Glu + Asn212Gln +
- 15 Thr214Ser  
 Ala194Thr + Gly196Asn + Val197Ser + Asn198Glu + Val199Asn  
 + Gln200Ser + Thr202Ser + Thr207Ser + Ser210Glu +  
 Gly213Ser
- 20 Gly196Pro + Val197Met + Asn198Asp + Val199Gly + Gln200Asn  
 + Tyr203Pro + Gly205Asn + Tyr208Val + Ser210Asp +  
 Leu211His
- Gly196Gln + Asn198Asp + Gln200Asn + Tyr203Asn + Gly205Pro  
 + Thr207Ser + Tyr208Gln + Ala209Pro + Leu211Glu +
- 25 Asn212Gln  
 Pro195Asn + Val197Pro + Asn198Glu + Val199Cys + Tyr203Asn  
 + Thr207Gly + Tyr208Gln + Ala209Ser + Leu211Glu +  
 Gly213Asn
- Ala194Gln + Gly196Gln + Val199Met + Gln200Asp + Tyr203Cys  
 + Thr207Ser + Ala209Asp + Asn212Ser + Gly213Gln +
- 30 Thr214Gln  
 Pro195Asn + Gly196Pro + Asn198Ser + Gln200Glu + Thr202Ser  
 + Thr207Gln + Tyr208Ala + Ala209Glu + Leu211Ala +  
 Asn212Gln
- 35 Pro195Gly + Gly196Asn + Val199Gln + Thr202Gly + Pro204Glu  
 + Gly205Gln + Ser206Glu + Thr207Ser + Leu211Gln +  
 Asn212Gln
- Pro195Gly + Gln200Asn + Tyr203Leu + Pro204Glu + Ser206Asp  
 + Tyr208Gln + Ala209Gln + Asn212Gln + Gly213Pro +
- 40 Thr214Gln  
 Ala194His + Pro195Ser + Val199Thr + Thr202Gly + Tyr203Cys  
 + Pro204Glu + Ser206Glu + Leu211His + Asn212Ser +  
 Gly213Pro
- Pro195Gln + Val197Gln + Asn198Gln + Gln200Ser + Pro204Glu  
 + Gly205Ser + Ser206Asp + Ala209Gly + Asn212Ser +
- 45 Thr214Gln  
 Ala194Thr + Asn198Asp + Val199Ala + Gln200Asn + Gly205Pro  
 + Tyr208Ser + Ala209Gly + Leu211Val + Asn212Glu +  
 Gly213Gln
- 50 Ala194Gly + Pro195Gln + Val199Cys + Tyr203Leu + Pro204Ser  
 + Gly205Glu + Thr207Asp + Ala209Gln + Asn212Gln +  
 Thr214Gly
- Pro195Gln + Val197Gln + Gln200Ser + Thr202Gln + Pro204Glu  
 + Gly205Ser + Thr207Asp + Leu211His + Asn212Ser +

- Thr214Ser  
Ala194Asn + Pro195Ser + Val197Ser + Asn198Gln + Thr202Asn  
+ Pro204Asp + Thr207Glu + Leu211His + Asn212Ser +  
Gly213Ser
- 5 Ala194Thr + Pro195Ser + Val197Gln + Asn198Glu + Val199Met  
+ Gln200Asp + Pro204Gly + Gly205Ser + Ala209Asn +  
Thr214Asn  
Pro195Gln + Gly196Pro + Val197Met + Asn198Asp + Val199Ala  
+ Gln200Glu + Thr202Pro + Tyr203Ile + Tyr208Cys +
- 10 Leu211Thr  
Ala194Gln + Gly196Asn + Asn198Glu + Val199Ala + Gln200Asp  
+ Thr202Ser + Tyr203Cys + Thr207Asn + Tyr208Met +  
Gly213Asn
- 15 Ala194Thr + Val197Pro + Val199His + Gln200Asp + Thr202Gln  
+ Tyr203Met + Gly205Ser + Thr207Asn + Tyr208Asp +  
Asn212Ser  
Pro195Asn + Val197Asp + Asn198Gln + Val199Gly + Gln200Ser  
+ Thr207Ser + Tyr208Ile + Ala209Asn + Asn212Ser +  
Gly213Asp
- 20 Pro195Asn + Val197Pro + Asn198Gln + Gln200Glu + Gly205Gln  
+ Tyr208Gly + Ala209Gly + Leu211Glu + Asn212Ser +  
Thr214Ser  
Ala194Thr + Gly196Ser + Asn198Ser + Gln200Asp + Thr202Gly  
+ Tyr203Met + Gly205Gln + Tyr208Ala + Leu211Asp +
- 25 Thr214Asn  
Pro195Ser + Asn198Ser + Val199Thr + Thr202Asn + Gly205Asn  
+ Thr207Ser + Ala209Gly + Leu211Glu + Asn212Gln +  
Gly213Asp  
Pro195Gln + Gly196Gln + Asn198Ser + Thr202Pro + Pro204Asn  
+ Thr207Asp + Tyr208Gly + Ala209Asp + Leu211Ile +
- 30 Asn212Ser  
Ala194Gly + Pro195Gly + Asn198Ser + Gln200Asn + Thr202Asn  
+ Tyr203Ala + Tyr208Val + Ser210Glu + Asn212Asp +  
Thr214Gly
- 35 Ala194Gln + Pro195Gln + Asn198Gln + Gln200Asn + Thr202Gly  
+ Tyr203Leu + Gly205Gln + Ala209Gln + Ser210Glu +  
Asn212Glu  
Pro195Gln + Val197Gly + Val199Gly + Thr202Asn + Gly205Pro  
+ Thr207Asn + Ala209Asn + Ser210Glu + Leu211Met +
- 40 Asn212Glu  
Ala194Thr + Pro195Gly + Gly196Pro + Asn198Ser + Thr207Pro  
+ Tyr208Cys + Ala209Ser + Ser210Glu + Leu211Ser +  
Asn212Asp
- 45 Ala194His + Pro195Ser + Gly196Gln + Val197Ala + Val199Asn  
+ Tyr203Gln + Thr207Asn + Tyr208His + Ser210Asp +  
Asn212Glu  
Ala194Gln + Pro195Asn + Gly196Ser + Val197Met + Pro204Ser  
+ Gly205Pro + Thr207Ser + Tyr208Ala + Ser210Glu +  
Asn212Glu
- 50 Ala194Ser + Val199Thr + Thr202Ser + Tyr203Ala + Pro204Asn  
+ Gly205Pro + Ser210Asp + Leu211Ala + Asn212Asp +  
Thr214Pro  
Pro195Asn + Thr202Gln + Pro204Ser + Thr207Gly + Tyr208Glu  
+ Ala209Asn + Ser210Glu + Leu211Asn + Asn212Gln +

- Gly213Asn  
 Gly196Asn + Val197Thr + Val199Thr + Pro204Ser + Thr207Gln  
 + Tyr208Asp + Ser210Asp + Leu211Thr + Asn212Gln +  
 Thr214Asn
- 5   Ala194Asn + Pro195Asn + Val199Cys + Gln200Asn + Pro204Gln  
 + Thr207Ser + Tyr208Glu + Ser210Asp + Leu211Asn +  
 Asn212Gln  
 Ala194Thr + Gly196Pro + Val199His + Gln200Ser + Gly205Asn  
 + Ser206Glu + Tyr208Glu + Ala209Thr + Leu211Met +
- 10   Thr214Ser  
 Ala194Ser + Pro195Gln + Asn198Gln + Val199His + Pro204Asn  
 + Gly205Gln + Ser206Glu + Tyr208Asp + Ala209Thr +  
 Gly213Asn  
 Gly196Gln + Val197Met + Val199Ser + Gln200Ser + Thr202Asn
- 15   + Pro204Asn + Tyr208Thr + Ala209Gln + Asn212Glu +  
 Thr214Asp  
 Val197Ala + Asn198Ser + Gln200Ser + Thr202Gly + Pro204Gln  
 + Thr207Gln + Tyr208Gln + Ala209His + Asn212Glu +  
 Thr214Asp
- 20   Ala194Pro + Gly196Ser + Val197Asp + Val199Asn + Thr202Asn  
 + Tyr203Val + Thr207Gln + Ala209Asn + Ser210Glu +  
 Asn212Gln  
 Pro195Ser + Val197Glu + Asn198Gln + Val199Gln + Tyr203Leu  
 + Tyr208Val + Ala209Asn + Asn212Gln + Gly213Pro +
- 25   Thr214Glu  
 Pro195Gln + Gly196Pro + Val197Asn + Asn198Glu + Gln200Ser  
 + Pro204Asn + Gly205Asn + Leu211Cys + Asn212Gln +  
 Gly213Asp  
 Pro195Gln + Gly196Pro + Val197Thr + Asn198Asp + Thr202Pro
- 30   + Tyr203Gln + Thr207Gly + Ala209Glu + Gly213Ser +  
 Thr214Ser  
 Ala194Ser + Pro195Gln + Val197Pro + Asn198Asp + Val199Ala  
 + Tyr203Thr + Pro204Gln + Ala209Asp + Leu211Val +  
 Asn212Ser
- 35   Pro195Gly + Val197Glu + Asn198Gln + Gln200Asp + Tyr203His  
 + Pro204Ser + Thr207Gly + Tyr208His + Gly213Pro +  
 Thr214Gly  
 Pro195Gly + Gly196Pro + Val199Thr + Gln200Glu + Tyr203Met  
 + Pro204Gln + Gly205Pro + Tyr208Ser + Asn212Asp +
- 40   Gly213Pro  
 Gly196Pro + Val197Ser + Val199Pro + Gln200Glu + Thr207Gly  
 + Ala209Gly + Leu211Gln + Asn212Asp + Gly213Pro +  
 Thr214Asn  
 Ala194His + Pro195Gln + Gly196Asn + Val197Ser + Gln200Glu
- 45   + Tyr203Ala + Pro204Asn + Gly205Ser + Thr207Pro +  
 Asn212Glu  
 Ala194Ser + Pro195Asn + Gly196Pro + Asn198Ser + Val199Thr  
 + Tyr208Ile + Leu211Glu + Asn212Gln + Gly213Ser +  
 Thr214Glu
- 50   Ala194Pro + Pro195Asn + Asn198Ser + Val199Met + Tyr203Met  
 + Gly205Pro + Thr207Pro + Tyr208Ser + Leu211Glu +  
 Thr214Glu  
 Ala194His + Pro195Gln + Gly196Gln + Val199Pro + Tyr203His  
 + Thr207Asn + Ala209Asp + Leu211Ala + Asn212Glu +

- Thr214Asn  
Ala194His + Gly196Gln + Thr202Ser + Tyr203Gly + Pro204Gly  
+ Gly205Ser + Ser206Asp + Thr207Gln + Ala209Asp +  
Thr214Pro
- 5 Pro195Gln + Thr202Ser + Pro204Gln + Gly205Pro + Ser206Asp  
+ Tyr208Met + Ala209Glu + Asn212Gln + Gly213Pro +  
Thr214Ser  
Gly196Gln + Val197Pro + Thr202Asn + Tyr203Gly + Gly205Pro  
+ Ser206Glu + Thr207Ser + Ala209Glu + Leu211Gln +
- 10 Asn212Ser  
Ala194Asn + Pro195Ser + Gly196Pro + Val199Ala + Thr202Gln  
+ Pro204Gln + Ser206Asp + Ala209Glu + Leu211His +  
Asn212Ser  
Gly196Ser + Asn198Ser + Val199Ser + Gln200Asn + Tyr203Asn
- 15 + Gly205Gln + Ser206Glu + Ala209Glu + Leu211Ser +  
Gly213Gln  
Ala194Thr + Val197Cys + Val199Asn + Pro204Gly + Ser206Asp  
+ Thr207Gly + Tyr208Gly + Ala209Glu + Leu211Ile +  
Thr214Gln
- 20 Ala194Gly + Pro195Ser + Gln200Ser + Thr202Gly + Tyr203Leu  
+ Pro204Gly + Gly205Pro + Ser206Asp + Ala209Asp +  
Thr214Gly  
Pro195Ser + Gly196Pro + Val199His + Gln200Asp + Thr202Pro  
+ Gly205Pro + Thr207Asp + Ala209Thr + Asn212Gln +
- 25 Gly213Gln  
Ala194Gln + Gly196Asn + Asn198Gln + Val199Pro + Gln200Asp  
+ Thr202Gly + Tyr203Thr + Gly205Pro + Thr207Asp +  
Ala209Gln  
Pro195Asn + Val197Thr + Val199Ala + Gln200Asn + Tyr203Asn
- 30 + Gly205Pro + Thr207Gly + Ser210Asp + Asn212Gln +  
Gly213Glu  
Ala194Asn + Pro195Gln + Val197Asn + Val199Asn + Pro204Gly  
+ Thr207Pro + Ser210Asp + Asn212Ser + Gly213Glu +  
Thr214Pro
- 35 Ala194Gln + Pro195Asn + Val197Gly + Tyr203Met + Pro204Ser  
+ Tyr208Gln + Ala209Asn + Ser210Glu + Asn212Ser +  
Gly213Glu  
Ala194His + Gly196Ser + Tyr203Asn + Pro204Gly + Gly205Pro  
+ Ala209Ser + Ser210Glu + Leu211Pro + Asn212Ser +
- 40 Gly213Asp  
Gly196Pro + Gln200Asn + Thr202Asn + Tyr203His + Gly205Asp  
+ Tyr208Ile + Ala209Glu + Leu211Met + Gly213Pro +  
Thr214Pro  
Val197His + Val199Asn + Gln200Asn + Thr202Ser + Tyr203Ser
- 45 + Gly205Glu + Tyr208Ser + Ala209Asp + Gly213Pro +  
Thr214Pro  
Ala194Asn + Val197Pro + Gln200Ser + Thr202Gln + Tyr203Met  
+ Gly205Gln + Thr207Asp + Ser210Asp + Leu211Ser +  
Asn212Ser
- 50 Ala194Thr + Asn198Ser + Tyr203Asn + Gly205Pro + Thr207Asp  
+ Ala209Gln + Ser210Glu + Leu211Val + Asn212Ser +  
Thr214Ser  
Asn198Gln + Val199Pro + Gln200Asn + Thr202Gln + Tyr203Asn  
+ Thr207Asp + Ala209Pro + Ser210Glu + Asn212Ser +

- Gly213Asn  
Ala194Ser + Pro195Asn + Asn198Gln + Val199Ala + Gln200Asp  
+ Tyr203His + Ala209Thr + Leu211Ser + Gly213Asp +  
Thr214Ser
- 5 Ala194Pro + Pro195Asn + Gly196Pro + Val197Ser + Gln200Asp  
+ Tyr208Cys + Leu211Gln + Asn212Ser + Gly213Asp +  
Thr214Gly  
Ala194Gln + Pro195Ser + Gly196Gln + Asn198Asp + Gln200Ser  
+ Thr202Ser + Tyr208Asp + Ala209Thr + Leu211His +
- 10 Thr214Asn  
Ala194Asn + Pro195Gln + Val197Thr + Asn198Asp + Pro204Asn  
+ Thr207Ser + Tyr208Asp + Leu211Ile + Gly213Gln +  
Thr214Gln  
Ala194Gln + Pro195Asn + Val199Asn + Tyr203Ser + Pro204Asp
- 15 + Thr207Asn + Ser210Asp + Leu211Gly + Asn212Ser +  
Gly213Ser  
Ala194Asn + Pro195Asn + Thr202Gly + Pro204Asp + Tyr208Ile  
+ Ala209Gly + Ser210Asp + Leu211Val + Asn212Gln +  
Thr214Ser
- 20 Ala194Asn + Gly196Ser + Asn198Ser + Val199Pro + Tyr203His  
+ Pro204Glu + Gly205Gln + Tyr208Met + Ala209Thr +  
Ser210Glu  
Ala194Thr + Asn198Ser + Val199Thr + Thr202Asn + Pro204Glu  
+ Gly205Asn + Thr207Gln + Ala209His + Ser210Glu +
- 25 Thr214Gly  
Ala194His + Pro195Gln + Asn198Ser + Val199Gly + Tyr203Gln  
+ Pro204Asp + Thr207Gly + Ser210Glu + Asn212Gln +  
Gly213Ser  
Pro195Asn + Tyr203Ser + Pro204Glu + Gly205Pro + Tyr208His
- 30 + Ala209Gly + Ser210Glu + Leu211Pro + Gly213Pro +  
Thr214Asn  
Gly196Asn + Asn198Ser + Gln200Ser + Thr202Gly + Pro204Asp  
+ Gly205Asn + Ala209Thr + Ser210Glu + Gly213Pro +  
Thr214Ser
- 35 Gln200Ser + Thr202Gln + Tyr203Met + Pro204Asp + Gly205Pro  
+ Tyr208Cys + Ala209Gly + Ser210Asp + Leu211Thr +  
Gly213Gln  
Ala194Pro + Gly196Asn + Gln200Ser + Tyr203Ser + Pro204Glu  
+ Thr207Gly + Ala209Asn + Ser210Asp + Leu211Val +
- 40 Thr214Ser  
Val199His + Gln200Ser + Thr202Ser + Pro204Asp + Gly205Asn  
+ Tyr208Pro + Ser210Asp + Leu211Pro + Gly213Pro +  
Thr214Pro  
Ala194Ser + Pro195Asn + Gly196Ser + Val197Ser + Asn198Gln
- 45 + Val199Ser + Pro204Glu + Tyr208Leu + Ser210Asp +  
Leu211Ser  
Ala194His + Gly196Pro + Val199Gly + Gln200Asp + Pro204Glu  
+ Tyr208Gln + Ala209Gly + Leu211Ser + Asn212Ser +  
Thr214Asn
- 50 Ala194Gln + Val197Pro + Val199Asn + Gln200Asp + Thr202Gln  
+ Pro204Asp + Gly205Asn + Tyr208His + Ala209Asn +  
Leu211Ile  
Pro195Ser + Val197Met + Asn198Ser + Gln200Asp + Thr202Gly  
+ Tyr203Gln + Pro204Glu + Thr207Pro + Asn212Ser +

- Thr214Gly  
 Pro195Ser + Val199Met + Gln200Ser + Pro204Gln + Thr207Ser  
 + Ala209Glu + Leu211Ala + Asn212Gln + Gly213Glu +  
 Thr214Gly
- 5 Pro195Gln + Gly196Pro + Asn198Ser + Val199Gly + Tyr203Asn  
 + Gly205Asn + Thr207Pro + Ala209Glu + Leu211Val +  
 Gly213Asp  
 Ala194Asn + Pro195Gln + Asn198Ser + Val199Ser + Gly205Asn  
 + Thr207Glu + Ala209Ser + Leu211Glu + Asn212Gln +
- 10 Thr214Ser  
 Ala194Ser + Asn198Ser + Gln200Ser + Thr202Gly + Tyr203Leu  
 + Thr207Gly + Ala209His + Ser210Glu + Asn212Ser +  
 Thr214Asp  
 Gly196Ser + Gln200Asn + Thr202Pro + Tyr203Leu + Pro204Asn  
 + Thr207Gln + Ser210Asp + Leu211Ala + Gly213Pro +
- 15 Thr214Asp  
 Gly196Ser + Asn198Gln + Tyr203Gly + Pro204Gln + Gly205Pro  
 + Thr207Pro + Tyr208His + Ser210Glu + Gly213Gln +  
 Thr214Asp
- 20 Val197Thr + Val199Thr + Gln200Ser + Thr202Ser + Gly205Asn  
 + Thr207Pro + Tyr208Cys + Ser210Glu + Gly213Ser +  
 Thr214Asp  
 Gly196Gln + Val197Thr + Gln200Asp + Thr202Asn + Tyr203His  
 + Ser206Glu + Thr207Ser + Tyr208Pro + Leu211Pro +
- 25 Asn212Gln  
 Val197Met + Val199Gln + Gln200Glu + Thr202Gln + Pro204Gln  
 + Gly205Asn + Ser206Asp + Thr207Gln + Leu211Val +  
 Thr214Ser  
 Ala194Thr + Pro195Gly + Gly196Asn + Val197Ser + Gln200Glu  
 + Thr202Pro + Gly205Pro + Ser206Glu + Leu211Met +
- 30 Asn212Ser  
 Pro195Ser + Gly196Gln + Val199Cys + Gln200Glu + Ser206Glu  
 + Tyr208Thr + Leu211Gln + Asn212Gln + Gly213Pro +  
 Thr214Asn
- 35 Gly196Gln + Val197Gly + Asn198Gln + Gln200Glu + Gly205Asn  
 + Ser206Glu + Thr207Pro + Ala209Asn + Asn212Gln +  
 Gly213Ser  
 Pro195Gln + Val197Gly + Gln200Glu + Thr202Gln + Tyr203Val  
 + Gly205Gln + Ser206Asp + Tyr208Met + Ala209Pro +
- 40 Thr214Gly  
 Ala194Ser + Pro195Asn + Val197Thr + Val199Thr + Gln200Glu  
 + Thr202Pro + Tyr203Ser + Gly205Pro + Ser206Glu +  
 Thr214Gly  
 Pro195Ser + Gln200Glu + Thr202Gln + Tyr203Gly + Gly205Pro  
 + Ser206Asp + Thr207Gly + Ala209His + Leu211His +
- 45 Thr214Gly  
 Ala194Asn + Pro195Gly + Gly196Asn + Val197Cys + Val199Asn  
 + Gln200Glu + Tyr203Pro + Pro204Gln + Ser206Asp +  
 Leu211Ala
- 50 Ala194Thr + Pro195Asn + Gly196Gln + Val197Asn + Gln200Glu  
 + Thr202Ser + Ser206Glu + Ala209His + Leu211Ala +  
 Thr214Ser  
 Val199Gly + Gln200Glu + Thr202Gln + Pro204Asn + Gly205Pro  
 + Ser206Asp + Thr207Ser + Ala209Gly + Leu211Cys +

Asn212Ser  
 Ala194Thr + Val197Ser + Val199Gln + Gln200Asp + Thr202Gln  
 + Pro204Gln + Ser206Glu + Ala209Gly + Leu211His +  
 Gly213Gln  
 5 Ala194Gly + Gly196Asn + Asn198Gln + Gln200Glu + Gly205Pro  
 + Ser206Asp + Ala209His + Leu211Pro + Gly213Gln +  
 Thr214Gly  
 Gly196Ser + Val199Ser + Thr202Ser + Pro204Asp + Thr207Asn  
 + Tyr208Leu + Leu211Glu + Asn212Gln + Gly213Ser +  
 10 Thr214Pro  
 Ala194Pro + Pro195Asn + Val197Pro + Thr202Pro + Tyr203Thr  
 + Thr207Gln + Tyr208Glu + Asn212Glu + Gly213Ser +  
 Thr214Ser  
 Gly196Asn + Gln200Ser + Gly205Pro + Thr207Gln + Tyr208Asp  
 15 + Ala209Ser + Leu211Ser + Asn212Glu + Gly213Pro +  
 Thr214Pro  
 Ala194Gly + Val199Pro + Gln200Asp + Thr202Pro + Tyr203Cys  
 + Pro204Gly + Ala209Gln + Leu211Cys + Asn212Gln +  
 Thr214Asp  
 20 Gly196Ser + Val197Gly + Gln200Asp + Tyr203Cys + Pro204Ser  
 + Thr207Gln + Leu211Ala + Asn212Gln + Gly213Ser +  
 Thr214Asp  
 Ala194Pro + Val197Thr + Thr202Gln + Tyr203Met + Pro204Asn  
 + Ser206Asp + Tyr208His + Ala209His + Ser210Asp +  
 25 Thr214Asn  
 Gly196Asn + Val197Cys + Thr202Asn + Tyr203Met + Gly205Pro  
 + Ser206Asp + Thr207Gly + Ala209Pro + Ser210Asp +  
 Gly213Gln  
 Ala194Thr + Val197Ser + Val199His + Thr202Gln + Tyr203Ser  
 30 + Ser206Asp + Thr207Ser + Ser210Asp + Gly213Pro +  
 Thr214Pro  
 Ala194Thr + Pro195Ser + Gly196Asn + Val197Pro + Asn198Gln  
 + Thr202Gln + Ser206Glu + Ser210Glu + Asn212Ser +  
 Thr214Gly  
 35 Pro195Asn + Gly196Ser + Val197Ser + Asn198Gln + Thr202Asn  
 + Tyr203Met + Ser206Glu + Ser210Asp + Asn212Gln +  
 Thr214Gln

TABLE 33

40	Multi-loop Double Mutation Variants
	Leu 94Gly + Gln200Glu
	Gln 57Ser + Asn 60Ser
	Val 93Gln + Gly213Asp
	Tyr102Cys + Thr207Gly
45	Ser154Glu + Asn198Gln
	Leu124Ile + Asn198Gln
	Ala209Gly + Ser210Glu
	Gln 57Asp + Leu 94Gly
	Leu 94Ala + Ser154Glu
50	Ser101Asp + Leu211Thr
	Ala131Glu + Gly196Gln
	Ser128Glu + Pro204Asn

	Ser103Asp + Pro129Asn
	Gly157Asn + Thr207Ser
	Gly 98Glu + Gly155Gln
	Gln200Asp + Ala209Pro
5	Asn 60Glu + Asn198Gln
	Leu 94Ile + Ser 99Asp
	Leu124Ile + Ser210Asp
	Ser126Glu + Thr202Ser
	Val 93Ala + Pro127Asp
10	Gly157Pro + Ser210Asp
	Val197Gly + Thr207Asp
	Gln185Glu + Ala194Thr
	Ser 97Glu + Tyr203Asn
	Gly100Pro + Thr207Asp
15	Ser210Asp + Leu211Met
	Tyr161Asn + Thr207Asp
	Leu124Ser + Pro127Asn
	Gly 61Ser + Thr207Pro
	Gly 98Glu + Gln200Ser
20	Gln200Asn + Asn212Glu
	Asp 58Glu + Ile105Cys
	Gly 61Pro + Ala209Thr
	Leu124Asp + Pro204Asn
	Gln 57Glu + Asn198Ser
25	Gln185Ser + Asn212Asp
	Phe183His + Val197Glu
	Asn 60Glu + Gln200Ser
	Gly 59Asn + Gly 61Glu
	Gly155Asp + Ile159Cys
30	Gly 63Gln + Leu211Gly
	Thr 64Pro + Tyr161Asp
	Leu 94Gln + Pro127Gly
	Thr207Gly + Gly213Glu
	Gln185Asp + Tyr203Ser
35	Gly 63Asn + Ala209Asn
	Tyr208Met + Gly213Asn
	Ser210Glu + Leu211Asn
	Tyr102Ala + Pro129Asn
	Gly125Glu + Leu211Gly
40	Ser103Asp + Thr207Ser
	Ser128Glu + Leu211His
	Gly155Gln + Leu211His
	Gln 57Asn + Gln200Asp
	Ser103Asp + Gly157Gln
45	Gly125Ser + Thr207Gly
	Ile105Pro + Val197Glu
	Pro204Asp + Ala209Gln
	Tyr161Asp + Thr207Asn
	Asn198Ser + Ser210Asp
50	Gly 95Ser + Gln200Asp
	Gly 59Gln + Ala156Glu
	Gly 98Gln + Tyr102Cys
	Ala181His + Thr207Glu
	Ser154Glu + Pro204Ser



	Gly205Asn + Thr207Gln
	Ser210Glu + Leu211Pro
	Leu 94Gln + Ser210Glu
	Ala209Asp + Leu211Pro
5	Asn 60Glu + Leu211His
	Ala 96Asn + Thr214Gly
	Asn 60Glu + Ala194Gly
	Ser103Asp + Ala181His
10	Gly125Glu + Ala209Asn
	Ser158Asp + Asn198Ser
	Gly155Ser + Asn198Ser
	Ser104Asp + Gly152Asn
	Ser182Asp + Leu211Cys
	Val 93Ser + Gly125Glu
15	Asn198Glu + Leu211Ser
	Gly100Ser + Ser103Asp
	Ala181Gln + Gly213Gln
	Thr 64Gly + Ser126Asp
	Ile159Cys + Gln200Asp
20	Thr207Glu + Leu211Asn
	Ser104Glu + Pro204Gly
	Ser130Asp + Pro204Asn
	Pro129Gln + Gln185Asn
	Asn 60Glu + Gly157Ser
25	Gly125Pro + Val197Met
	Asn 60Glu + Ile105Cys
	Pro127Gln + Val197Thr
	Ser101Asp + Leu211Ala
	Gln 57Ser + Ser 99Glu
30	Phe183Pro + Asn198Glu
	Asn198Glu + Thr202Pro
	Val197Gln + Leu211His
	Gly 61Ser + Gln200Ser
	Gly 61Asp + Asn198Gln
35	Gly 61Glu + Phe183Ser
	Thr207Asn + Asn212Glu
	Tyr203Thr + Tyr208Gly
	Asn198Ser + Leu211Cys
	Asn153Asp + Leu211Ser
40	Gln 57Asn + Gly213Pro
	Thr 64Ser + Ser210Asp
	Pro204Asp + Leu211Ile
	Gln 57Asp + Gly 61Pro
	Gly155Gln + Ser210Asp
45	Ala156Gly + Thr207Asn
	Gly 61Gln + Ala181Thr
	Ile159Cys + Asn198Gln
	Pro129Gly + Leu211Glu
	Asn198Glu + Thr207Ser
50	Gly 98Ser + Ser128Asp
	Ala131Asn + Val193Ser
	Gln185Ser + Gln200Asn
	Ser210Asp + Leu211His
	Gly125Glu + Ala156Gln

100

	Ser 97Asp + Gly125Gln
	Asn 60Gln + Gly155Pro
	Ser126Glu + Thr207Ser
	Phe183Met + Thr207Pro
5	Ser154Glu + Phe183Ile
	Val 93Gln + Ser210Asp
	Val 93Gln + Tyr208His
	Asn 60Ser + Asn198Glu
	Gly152Gln + Gln200Asp
10	Pro127Asn + Gly152Asn
	Gly155Gln + Thr214Asn
	Ala 96Ser + Tyr208His
	Tyr161Thr + Ser210Asp
	Gly100Pro + Pro204Gln
15	Gln 57Asp + Ala194Asn
	Asn 60Gln + Thr202Asn
	Gly157Pro + Gly213Asn
	Val 93Thr + Leu211Ala
	Ser103Glu + Leu211Thr
20	Ser101Asp + Leu124Ile
	Leu 94Glu + Pro204Gln
	Gln200Glu + Ala209His
	Ser101Glu + Gly152Gln
	Asn198Asp + Gly205Pro
25	Gly 61Ser + Gln200Asn
	Ser158Asp + Val197Asn
	Gly 61Glu + Phe183Ile
	Asp 58Glu + Tyr203Ser
	Gly213Ser + Thr214Gly
30	Gln 57Asp + Asn198Gln
	Tyr102Leu + Ser158Glu
	Ala 96Gln + Leu211Asn
	Val 93Asp + Thr202Asn
	Leu 94Ser + Asn198Asp
35	Gly196Ser + Asn212Asp
	Gly155Asn + Gly157Asp
	Val 93Asn + Ser130Asp
	Leu 94Ile + Ser210Asp
	Val197Ala + Ser210Asp
40	Ser104Asp + Gly205Pro
	Asn153Asp + Ala181Gly
	Gln200Asn + Leu211Glu
	Leu 94Asn + Ser210Glu
	Gln185Asp + Val197Gln
45	Tyr102Ser + Thr207Asp
	Gly 61Glu + Gly 95Asn
	Gly 61Asp + Pro129Gln
	Ser 99Asp + Thr207Ser
	Ser126Glu + Gly152Gln
50	Val197His + Ser206Glu
	Gly 61Asn + Gly155Glu
	Gly155Asn + Asn198Glu
	Ala209Asn + Ser210Glu
	Ser128Asp + Thr207Gly

WO 95/30011

101

5 Ala209Thr + Ser210Asp  
 Gln185Asn + Gly213Pro  
 Ser126Glu + Leu211Gly  
 Ser 97Asp + Gly205Ser  
 Leu 94Thr + Leu124Thr  
 Asn 60Gln + Leu211Gly  
 Pro129Asp + Gly155Pro  
 Asp 58Glu + Gly157Ser  
 Thr202Pro + Thr207Glu  
 10 Ala156Ser + Tyr208Asp  
 Gly 98Glu + Tyr161Gln  
 Ile105Cys + Gln200Asn  
 Gln200Glu + Pro204Gly  
 Gly 98Pro + Ala131Asn  
 Gly 59Asp + Thr207Gln  
 15 Tyr102Gln + Ala209Gln  
 Gly 98Glu + Ile105His  
 Ser101Asp + Pro129Gln  
 Pro127Asp + Gly213Asn  
 Ser104Glu + Asn198Gln  
 20 Gln 57Ser + Gln200Asn  
 Val197Cys + Gln200Ser  
 Val 93Ala + Ala156Glu  
 Gln200Asn + Gly205Glu  
 Leu 94Ala + Thr207Asn  
 25 Ala131Thr + Gln200Ser  
 Leu 94Glu + Tyr161Thr  
 Gln200Asn + Ser210Asp  
 Gln 57Asp + Thr207Asn  
 Ser 99Glu + Ala156Pro  
 30 Leu124Ile + Asn198Ser  
 Tyr161Gly + Thr207Gln  
 Ile105Cys + Thr214Asp  
 Ile159His + Ser206Asp  
 Gly 63Gln + Gly125Asn  
 35 Asn198Asp + Gly205Ser  
 Ser104Glu + Leu124Gln  
 Ala209Pro + Ser210Glu  
 Tyr161Val + Asn198Asp  
 Pro129Ser + Gln200Asp  
 40 Ser 97Asp + Tyr203Ser  
 Ser103Asp + Phe183Thr  
 Tyr161Gly + Val197Ala  
 Asn198Gln + Pro204Glu  
 Leu211Gly + Asn212Glu  
 45 Ser206Glu + Leu211Thr  
 Gly152Pro + Val197Ser  
 Ile105Ala + Ala156Gln  
 Gly 63Asn + Tyr102Pro  
 Ser182Glu + Val197Asn  
 50 Thr 64Glu + Ala 96Gln  
 Asn 60Gln + Ser206Glu  
 Asn 60Gln + Ser128Asp  
 Ser101Asp + Ile159Pro

	Leu124Cys + Asn153Ser
	Asn198Asp + Leu211Pro
	Ser126Glu + Tyr208Met
5	Ala 96Asn + Gly100Ser
	Ser 99Glu + Leu211Cys
	Ser 97Asp + Leu211Ala
	Ser182Glu + Leu211His
	Ile159Cys + Leu211Glu
10	Gly152Asn + Ser210Asp
	Val197Ala + Gln200Glu
	Asn 60Ser + Ser158Glu
	Ser104Glu + Tyr208Asn
	Pro204Asp + Leu211Gly
	Asp 58Glu + Val197Gly
15	Ser182Glu + Tyr208Ser
	Ser103Asp + Ala194Ser
	Ala156Gln + Thr207Asp
	Asn198Ser + Thr207Ser
	Gln 57Asp + Thr207Ser
20	Gly152Glu + Thr207Gly
	Ala131Ser + Ile159Asn
	Pro127Asn + Gly152Glu
	Gln200Asn + Gly213Glu
	Leu124Asp + Leu211Ala
25	Gly 98Glu + Gly125Asn
	Ile159Gly + Thr207Asn
	Pro127Gln + Asn153Asp
	Ser103Glu + Phe183Tyr
30	Ser160Glu + Pro204Ser
	Gly157Ser + Tyr161His
	Ala156Pro + Ser158Glu
	Gly100Glu + Pro127Asn
	Gln 57Asn + Asn 60Glu
	Gly125Ser + Ser210Glu
35	Gly125Pro + Ser128Asp
	Gly125Glu + Val197Gly
	Val193Met + Asn198Ser
	Ser160Glu + Gly213Asn
	Ser206Glu + Ala209His
40	Ser 97Glu + Gly213Ser
	Gly100Asp + Leu211Gly
	Pro129Gln + Thr207Gly
	Gly 95Gln + Ser210Asp
	Gln185Ser + Pro204Glu
45	Pro129Glu + Ala156Ser
	Ser126Asp + Asn212Ser
	Gly 59Ser + Ile159Ala
	Ala 96Asn + Ser 99Glu
	Ser104Asp + Ile159Val
50	Gly 63Asn + Ser210Glu
	Phe183Pro + Ser210Glu
	Ala 96Asp + Tyr208Ile
	Gly 98Asp + Thr207Gln
	Val 93Gln + Gly125Glu

104

	Ile159Glu + Thr207Gln
	Leu 94Ile + Leu211Val
	Gly157Asp + Leu211His
	Gly 61Asp + Leu211Cys
5	Gln 57Asn + Gly157Asn
	Gly100Glu + Val197Ser
	Gln200Glu + Pro204Asn
	Ala131Gly + Leu211His
	Ser206Asp + Leu211Asn
10	Asn153Asp + Ile159Leu
	Gly152Ser + Asn198Glu
	Leu 94Met + Ser130Glu
	Gln 57Glu + Asn198Gln
	Ser182Glu + Leu211Thr
15	Val199Ala + Ser210Glu
	Thr207Gly + Ser210Glu
	Thr 64Gly + Gly 98Asp
	Gly 61Gln + Ser 99Asp
	Gly155Glu + Leu211Ser
20	Leu124Ser + Thr207Asp
	Val 93Ser + Asn198Gln
	Ser 99Asp + Gly125Asn
	Gln 57Asn + Asn198Glu
	Ser 99Glu + Ile159Met

25

TABLE 34

## Multi-loop Triple Mutation Variants

	Gln 57Ser + Leu 94Gly + Gln200Glu
	Asn 60Ser + Val 93Gln + Gly213Asp
30	Tyr102Cys + Asn198Gln + Thr207Gly
	Leu124Ile + Ser154Glu + Asn198Gln
	Leu 94Gly + Ala209Gly + Ser210Glu
	Gly155Asp + Ala209Gln + Asn212Gln
	Pro129Asn + Gly157Asn + Thr207Ser
35	Asn198Gln + Gln200Asp + Ala209Pro
	Val 93Ala + Pro127Asp + Thr202Ser
	Tyr161Asn + Thr207Asp + Leu211Met
	Gly 61Ser + Pro127Asn + Thr207Pro
	Gly 61Pro + Leu124Asp + Pro204Asn
40	Gln 57Glu + Gln185Ser + Asn198Ser
	Gly 59Asn + Asn 60Glu + Gln200Ser
	Gly 63Gln + Gln200Asp + Leu211Gly
	Tyr203Ser + Thr207Gly + Gly213Glu
	Gly 63Asn + Gln185Asp + Ala209Asn
45	Pro129Asn + Ser210Glu + Leu211Asn
	Tyr102Ala + Gly125Glu + Leu211Gly
	Gln 57Asn + Gly155Gln + Leu211His
	Ile105Pro + Gly125Ser + Thr207Gly
	Asn198Ser + Thr207Asn + Ser210Asp
50	Gly 59Gln + Gly 95Ser + Ala156Glu
	Tyr102Cys + Ser210Glu + Leu211Asn
	Gly205Asn + Thr207Gln + Ser210Glu

Leu 94Gln + Ala209Asp + Leu211Pro  
Asn 60Glu + Leu211His + Thr214Gly  
Asn 60Glu + Ala181His + Ala194Gly  
Ser104Asp + Gly155Ser + Asn198Ser  
5 Gly152Asn + Ser182Asp + Leu211Cys  
Gly100Ser + Ser103Asp + Leu211Ser  
Thr 64Gly + Ser126Asp + Ile159Cys  
Ser130Asp + Ala181Asn + Pro204Gly  
Pro129Gln + Gly157Ser + Gln185Asn  
10 Asn 60Glu + Gly125Pro + Val197Met  
Ile105Cys + Pro127Gln + Val197Thr  
Gln 57Ser + Ser 99Glu + Phe183Pro  
Gly 61Ser + Val197Gln + Leu211His  
Gly 61Asp + Asn198Gln + Gln200Ser  
15 Tyr203Thr + Thr207Asn + Tyr208Gly  
Asn153Asp + Asn198Ser + Leu211Ser  
Gln 57Asn + Ser210Asp + Gly213Pro  
Thr 64Ser + Pro204Asp + Leu211Ile  
Ser101Asp + Ala156Gly + Thr207Asn  
20 Ile159Cys + Ala181Thr + Asn198Gln  
Gly 98Ser + Asn198Glu + Thr207Ser  
Ser128Asp + Ala131Asn + Val193Ser  
Gln185Ser + Gln200Asn + Leu211His  
Ser126Glu + Phe183Met + Thr207Ser  
25 Ser154Glu + Phe183Ile + Thr207Pro  
Val 93Gln + Tyr208His + Ser210Asp  
Val 93His + Pro127Asn + Gly152Gln  
Gly155Gln + Gly213Glu + Thr214Asn  
Gly100Pro + Tyr161Thr + Ser210Asp  
30 Gln 57Asp + Ala194Asn + Pro204Gln  
Gly157Pro + Thr202Asn + Gly213Asn  
Thr 64Gln + Val 93Thr + Leu211Ala  
Gly 61Ser + Gln200Asn + Gly205Pro  
Ser158Asp + Phe183Ile + Val197Asn  
35 Asn198Asp + Gly213Ser + Thr214Gly  
Gly152Ser + Gly155Ser + Ser206Asp  
Gly152Gln + Gln185Ser + Val197Gln  
Leu 94Ile + Val197Ala + Ser210Asp  
Ala181Gly + Gln200Asn + Leu211Glu  
40 Leu 94Asn + Val197Gln + Ser210Glu  
Gly 61Asp + Pro129Gln + Thr207Ser  
Gln 57Asn + Gly 61Asn + Gly155Glu  
Gly 61Pro + Ser128Asp + Ala209Thr  
Thr207Gly + Ala209Thr + Ser210Asp  
45 Ser128Asp + Gln185Asn + Gly213Pro  
Leu 94Thr + Leu124Thr + Gly205Ser  
Asn 60Gln + Gly155Pro + Leu211Gly  
Asp 58Glu + Gly157Ser + Thr202Pro  
Gly 98Glu + Ile105Cys + Gln200Asn  
50 Gly 98Pro + Gln200Glu + Pro204Gly  
Gly 59Asp + Ala131Asn + Thr207Gln  
Gly 98Glu + Tyr102Gln + Ala209Gln  
Ser101Asp + Ile105His + Pro129Gln  
Pro127Asp + Asn198Gln + Gly213Asn

5 Ile159Gly + Val197Gly + Asn198Gln  
 Gln 57Ser + Gly155Glu + Gln200Asn  
 Val197Cys + Asn198Glu + Gln200Ser  
 Leu 94Ala + Gln200Asn + Gly205Glu  
 Ala131Thr + Gln200Ser + Thr207Asn  
 10 Leu124Ile + Ala156Pro + Asn198Ser  
 Val 93Glu + Tyr161Gly + Thr207Gln  
 Gly 63Gln + Gly125Asn + Ala209Glu  
 Tyr161Gly + Val197Ala + Asn198Gln  
 Ser158Asp + Asn198Ser + Leu211Gly  
 15 Ile105Ala + Gly152Pro + Val197Ser  
 Gly 63Asn + Tyr102Pro + Ala156Gln  
 Ala 96Gln + Ser182Glu + Val197Asn  
 Asn 60Gln + Ser101Asp + Ile159Pro  
 Gly100Ser + Gly205Ser + Thr207Ser  
 20 Tyr161Ser + Ala181Glu + Thr207Asn  
 Gly125Ser + Gln200Glu + Leu211Ala  
 Gly 59Asp + Tyr102Thr + Thr207Pro  
 Gly 59Ser + Gly100Gln + Tyr102Met  
 Asn 60Ser + Leu 94Cys + Asn198Asp  
 25 Pro127Glu + Ala156Asn + Gly205Pro  
 Asp 58Glu + Ala181Thr + Gly205Ser  
 Thr 64Gln + Val 93Ala + Asn198Ser  
 Phe183Asp + Leu211Thr + Asn212Gln  
 Pro127Asn + Ser130Asp + Phe183Thr  
 Tyr161Gln + Tyr208Ile + Leu211Cys  
 Leu124Ser + Asn153Gln + Gly213Asn  
 Val 93Thr + Ser101Glu + Asn212Gln  
 30 Tyr161Gln + Gln200Asn + Gly213Ser  
 Gln200Asp + Thr207Pro + Leu211Asn  
 Thr 64Pro + Leu124Val + Ser126Glu  
 Thr 64Ser + Ser 97Glu + Asn198Ser  
 Gly152Gln + Ser210Asp + Leu211Pro  
 Val 93Pro + Asn198Ser + Gln200Glu  
 35 Thr 64Gln + Tyr102Leu + Asn153Ser  
 Ala 96Asn + Gly100Ser + Tyr208Met  
 Ile159Cys + Ser182Glu + Leu211His  
 Asn 60Ser + Ser158Glu + Tyr208Asn  
 Ser103Asp + Ala194Ser + Tyr208Ser  
 40 Ala156Gln + Asn198Ser + Thr207Asp  
 Ala131Ser + Gly152Glu + Ile159Asn  
 Pro127Asn + Gln200Asn + Gly213Glu  
 Gly 98Glu + Gly125Asn + Leu211Ala  
 Ile159Gly + Thr207Asn + Ser210Glu  
 45 Ser103Glu + Phe183Tyr + Pro204Ser  
 Gly157Ser + Ser160Glu + Tyr161His  
 Gln 57Asn + Gly100Glu + Pro127Asn  
 Gly125Pro + Val197Gly + Ser210Glu  
 Val193Met + Asn198Ser + Gly213Asn  
 50 Gly 95Gln + Pro129Gln + Thr207Gly  
 Gly 59Ser + Ser126Asp + Asn212Ser  
 Gly 63Asn + Ile159Val + Ser210Glu  
 Phe183Pro + Tyr208Ile + Ser210Glu  
 Leu 94Ile + Ile159Glu + Thr207Gln

5 Gln 57Asn + Gly157Asn + Leu211Cys  
 Ala131Gly + Pro204Asn + Leu211His  
 Ile159Leu + Ser206Asp + Leu211Asn  
 Leu 94Met + Gly152Ser + Asn198Glu  
 Ser182Glu + Val199Ala + Leu211Thr  
 Leu124Ser + Thr207Asp + Leu211Ser  
 Val 93Ser + Gly125Asn + Asn198Gln  
 Tyr102Pro + Ala156Ser + Phe183His  
 10 Gly 98Gln + Ser128Glu + Ile159Cys  
 Ser104Asp + Ile105Pro + Tyr161Thr  
 Tyr102Pro + Ile105Thr + Ala181Glu  
 Thr 64Ser + Ile105Glu + Gln200Asn  
 Ser104Asp + Gln200Ser + Thr214Pro  
 Thr 64Pro + Ala156Glu + Thr202Gly  
 15 Gly 63Gln + Gly100Asp + Leu211His  
 Asn 60Ser + Gly 98Asp + Leu211Thr  
 Tyr161Gly + Val197Cys + Ser206Glu  
 Thr207Gln + Tyr208Gln + Ser210Glu  
 Gly 98Ser + Ile105Leu + Ser210Glu  
 20 Thr 64Gly + Pro195Ser + Thr214Ser  
 Val 93Met + Ser154Asp + Leu211Met  
 Leu124Ser + Asn198Ser + Leu211Asp  
 Thr 64Gly + Ala156Ser + Gly157Pro  
 Gly 59Ser + Gln200Asn + Gly213Glu  
 25 Gly 61Asn + Leu124Ile + Ala181Pro  
 Ala 96His + Gln185Asp + Leu211Ala  
 Ser101Asp + Asn198Ser + Thr214Ser  
 Leu 94Met + Asn153Asp + Pro195Gln  
 Pro127Gln + Gly152Asp + Val193His  
 30 Ser 97Asp + Ile105Thr + Thr207Gln  
 Gly 61Pro + Ser160Glu + Thr207Gln  
 Leu124Val + Pro204Gly + Leu211Ile  
 Ser 99Asp + Asn198Gln + Gln200Ser  
 Ser101Glu + Ala181Asn + Ala209Pro  
 35 Gly 61Asn + Ala 96Thr + Gly 98Ser  
 Val 93Pro + Asn198Asp + Thr207Gln  
 Thr 64Pro + Ala 96Pro + Gly155Glu  
 Thr 64Gly + Ala181Thr + Thr214Pro  
 Thr 64Pro + Leu 94Cys + Tyr208Met  
 40 Gln 57Asn + Leu 94Gln + Thr214Asn  
 Leu 94Gln + Tyr161Pro + Thr207Gly  
 Val 93His + Gln185Asn + Thr207Pro  
 Gly125Pro + Asn198Glu + Gln200Ser  
 Asn153Ser + Ile159Cys + Ser160Asp  
 45 Thr 64Gly + Gln200Glu + Leu211Cys  
 Leu 94Val + Pro195Gln + Leu211Asp  
 Gly 59Pro + Gly100Ser + Thr207Pro  
 Tyr102Cys + Gly157Gln + Thr207Pro  
 Ser 99Asp + Leu124Ser + Thr214Asn  
 50 Gly 59Gln + Gln200Asp + Thr207Ser  
 Ala 96His + Ala181Asp + Leu211Cys  
 Val 93Met + Ala181His + Thr207Ser  
 Pro129Gln + Asn198Ser + Thr207Ser  
 Asp 58Glu + Gly 95Ser + Ala194Gly



	Ser160Glu + Thr207Gln + Thr214Gly
	Thr 64Asn + Phe183Asp + Thr207Asn
	Ser158Asp + Phe183Ile + Val197His
	Gly 95Pro + Asn153Ser + Gln200Glu
5	Gly157Asp + Pro204Gly + Thr207Ser
	Val197Pro + Asn198Gln + Thr207Gln
	Gly 61Pro + Leu 94Glu + Tyr161Ile
	Pro129Gln + Gly155Pro + Ser210Glu
	Val 93Met + Ser182Asp + Gln200Ser
10	Pro127Ser + Leu211Met + Gly213Ser
	Ala 96His + Ser160Asp + Tyr208Gly
	Gly 98Pro + Pro129Ser + Pro204Asp
	Gly 59Ser + Gly 63Pro + Pro129Asp
	Gly100Pro + Leu124Pro + Thr207Asn
15	Ser103Asp + Ile159Ala + Thr202Gln
	Gly 59Asn + Ser130Glu + Leu211Thr
	Ala 96Gly + Ala131Asn + Gly155Pro
	Asn153Ser + Gln200Asn + Ser210Glu
	Asn153Glu + Thr207Pro + Leu211Ala
20	Pro127Asn + Val197His + Thr207Asp
	Gly100Asp + Ala131Gln + Tyr208Thr
	Gly125Pro + Phe183Pro + Asn198Glu
	Asn 60Ser + Ile105Thr + Thr207Glu
	Thr 64Ser + Pro127Asp + Ala131Thr
25	Ser 99Asp + Leu124Asn + Phe183Pro
	Thr 64Gln + Ser101Asp + Leu211Ser
	Pro195Gln + Val197Gly + Leu211Ser
	Gly100Asn + Tyr102Gly + Thr207Pro
	Asn198Ser + Gln200Asp + Leu211Pro
30	Tyr102Met + Ser182Glu + Leu211Ala
	Gly 61Asn + Gln200Asn + Gly213Asn
	Ser 99Asp + Gly152Pro + Gly157Ser
	Leu124Ser + Tyr161Met + Gln200Glu
	Tyr102Met + Leu211Glu + Thr214Ser
35	Asp 58Glu + Gly 98Asn + Tyr161Asn
	Ile159Ser + Gln200Asn + Tyr208Asn
	Thr 64Asn + Ala156Gly + Leu211Asp
	Gln200Asn + Ser210Glu + Leu211Thr
	Asn 60Asp + Gly155Pro + Val197Asn
40	Ala156Gln + Asn198Glu + Gly205Gln
	Gln 57Asp + Gln200Ser + Tyr208Thr
	Leu124Ile + Ala209Glu + Ser210Asp
	Gly 59Glu + Asn 60Asp + Leu211Ala
	Ala131Gly + Gly152Asp + Asn153Glu
45	Tyr203Val + Ser210Glu + Leu211Glu
	Gly152Asn + Ser210Asp + Leu211Glu
	Phe183Val + Ser210Asp + Leu211Asp
	Tyr102Leu + Ser210Asp + Leu211Glu
	Gln200Asn + Thr207Glu + Tyr208Glu
50	Ala156Ser + Thr207Glu + Tyr208Asp
	Pro129Asp + Ser130Asp + Asn198Ser
	Asn198Ser + Gln200Glu + Ser210Asp
	Leu124Cys + Gln200Glu + Ser210Glu
	Thr 64Gly + Gln200Asp + Ser210Asp

5 Gly 61Asn + Gln200Asp + Ser210Glu  
Phe183His + Val197Glu + Asn212Asp  
Ala 96Gly + Ser101Glu + Ser103Glu  
Ser126Glu + Ser128Glu + Asn198Ser  
Gly 61Pro + Ser154Asp + Ala156Glu  
Val 93Asp + Ser104Asp + Leu211Ala  
Ala 96Ser + Gly125Asp + Tyr161Asp  
Ser 97Asp + Ser 99Glu + Leu211Cys  
Ser 97Asp + Ser 99Glu + Asn153Gln  
10 Ala 96Pro + Gly152Asp + Ser154Asp  
Gly 98Asn + Asn198Asp + Gln200Asp  
Asn 60Ser + Asn198Glu + Gln200Asp  
Pro129Ser + Asn198Asp + Gln200Asp  
Gln 57Glu + Gly 59Glu + Leu211Gln  
15 Ser128Asp + Ser130Glu + Ala209His  
Ser158Asp + Ser160Glu + Gln185Asp  
Pro127Gln + Ser210Glu + Asn212Asp  
Ser210Glu + Leu211Asn + Asn212Asp  
Val197Asp + Gln200Glu + Ser210Asp  
20 Phe183Glu + Tyr208Val + Gly213Asp  
Pro127Glu + Gly152Glu + Tyr161Asp  
Leu 94Glu + Ser101Asp + Leu124Ile  
Leu 94Glu + Ser101Asp + Gln200Asn  
Pro127Gln + Asn153Glu + Ala156Glu  
25 Asp 58Glu + Gly 61Gln + Gly 95Asp  
Pro129Gly + Val197Asp + Ser210Glu  
Asp 58Glu + Gly 61Glu + Gly 95Glu  
Val197Glu + Ser210Asp + Gly213Asp  
Ser154Glu + Ser182Glu + Pro204Ser  
30 Ser101Glu + Gly125Glu + Ser126Glu  
Ser 99Asp + Ser104Asp + Ile105Leu  
Ser128Glu + Ala131Glu + Gly196Gln  
Gly 61Glu + Ser 97Asp + Ala131His  
Ser 97Asp + Gly100Asp + Ser104Glu  
35 Asp 58Glu + Pro204Asn + Gly205Asp  
Gln185Glu + Leu211Cys + Gly213Asp  
Gln200Asp + Thr207Glu + Leu211Asn  
Gly125Ser + Gln200Asp + Thr207Glu  
Gln200Glu + Thr207Asp + Leu211Cys  
40 Asp 58Glu + Val 93Gly + Ser 97Glu  
Asp 58Glu + Ser 97Glu + Tyr203Asn  
Gly 61Glu + Gly 95Asn + Thr207Asp  
Ala 96Pro + Thr207Asp + Ser210Asp  
Pro204Asn + Thr207Glu + Ser210Asp  
45 Gly100Pro + Thr207Asp + Ser210Asp  
Gly 61Asp + Thr207Asp + Ser210Asp  
Asp 58Glu + Thr 64Asp + Ser 97Glu  
Asn153Asp + Gly157Glu + Ala181Glu  
Ser130Asp + Ser160Asp + Gly205Gln  
50 Tyr102Asp + Ser128Asp + Ser160Asp  
Gln 57Asp + Ser206Asp + Thr207Glu  
Ser 99Asp + Ser126Glu + Gly152Gln  
Ala181His + Asn198Glu + Thr214Glu  
Gln 57Asn + Thr 64Glu + Gln200Glu

	Thr 64Asp + Leu124Thr + Gln200Asp
	Gly 98Asp + Gly125Glu + Thr207Gln
	Asn198Glu + Gln200Glu + Pro204Glu
	Asp 58Glu + Gly 59Asp + Gln200Asp
5	Leu124Asp + Val197Asp + Asn198Asp
	Pro129Glu + Asn212Asp + Gly213Glu
	Ser158Asp + Ser210Asp + Leu211Glu
	Ser101Glu + Tyr102Glu + Gln200Asp
	Ser103Asp + Ser104Asp + Asn198Asp
10	Asn 60Glu + Ser103Glu + Ser104Glu
	Ser182Asp + Gln200Asp + Ser210Asp
	Gly157Glu + Gln200Glu + Ser210Asp
	Gln 57Asp + Gln200Asp + Ser210Glu
	Ser 99Glu + Gln200Asp + Ser210Asp
15	Ser182Glu + Asn198Asp + Gln200Glu
	Gln 57Ser + Gln200Asp + Gly213Glu
	Ser 99Glu + Ser103Asp + Ile159Met
	Ser 99Asp + Ser103Asp + Gly213Gln
	Gly155Glu + Gln185Glu + Asn198Glu
20	Gly155Asp + Gln185Glu + Ser210Asp
	Gln 57Asp + Thr 64Glu + Ser210Glu
	Thr207Glu + Leu211Glu + Gly213Asp
	Ser126Asp + Ser160Asp + Ser210Asp
	Ser130Glu + Asn198Asp + Ser210Asp
25	Ser126Asp + Asn198Asp + Ser210Glu
	Ser101Asp + Asn198Glu + Leu211Glu
	Gly155Glu + Asn198Asp + Leu211Glu
	Asp 58Glu + Asn 60Glu + Ser103Asp
	Ser 99Glu + Ser104Glu + Ser126Glu
30	Gly 59Asp + Gly 61Gln + Ser 99Asp
	Asn 60Glu + Ser158Glu + Gln185Glu
	Gly100Glu + Ser104Asp + Ser210Glu
	Gly100Asp + Ser104Asp + Ser182Glu
	Ser128Glu + Ser130Asp + Asn198Glu
35	Ser154Glu + Gln200Asp + Leu211Glu
	Ser103Asp + Gln200Glu + Leu211Asp
	Ser103Glu + Gln200Glu + Leu211Asp
	Ser126Asp + Ala156Glu + Ser182Asp
	Ser158Asp + Ser160Glu + Val197Glu
40	Ser158Asp + Ser160Asp + Val197Glu
	Val 93Gln + Gly125Glu + Ser158Glu
	Gln 57Asp + Leu124Asp + Ser126Asp
	Asn 60Asp + Ser210Glu + Asn212Glu
	Gly157Asp + Ser210Glu + Asn212Asp
45	Ser 99Glu + Ser101Glu + Ser210Glu
	Asp 58Glu + Asn198Glu + Pro204Glu
	Tyr102Asp + Ser130Glu + Ser182Asp
	Asn 60Asp + Gly 95Glu + Ser128Asp
	Leu 94Glu + Ser 97Asp + Ser206Glu
50	Gln 57Asp + Ser126Glu + Gly152Asp
	Leu 94Glu + Ser101Glu + Leu211Asp
	Gly 59Glu + Ser 97Glu + Ser182Glu
	Gly100Asp + Ser103Glu + Ser206Asp
	Asn 60Glu + Gln185Glu + Thr214Glu

111

	Asn 60Glu + Ser130Glu + Pro204Asp
	Asn 60Asp + Asn198Asp + Pro204Asp
	Ser 99Asp + Ser182Glu + Gln185Asp
	Pro204Asp + Ser210Glu + Leu211Gly
5	Tyr161Val + Pro204Glu + Ser210Glu
	Ser101Glu + Asn198Asp + Gly213Glu
	Leu124Asp + Ser154Glu + Val193Ser
	Ser 99Glu + Ala181Glu + Asn212Asp
	Ala 96Asp + Gly100Asp + Ser154Glu
10	Gly 61Asp + Ser 99Asp + Ser210Glu
	Asp 58Glu + Thr207Glu + Ser210Asp

TABLE 35

Multi-loop Quadruple Mutation Variants	
15	Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu
	Val 93Gln + Tyr102Cys + Thr207Gly + Gly213Asp
	Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln
	Leu 94Gly + Ser103Glu + Gln200Asn + Ala209Gly
	Ala156His + Gln200Ser + Thr207Pro + Leu211Thr
20	Pro129Asn + Gly155Asp + Ala209Gln + Asn212Gln
	Leu124Pro + Ala194Asn + Asn198Glu + Gln200Asn
	Gly 61Ser + Asn198Gln + Gln200Ser + Leu211His
	Tyr203Thr + Thr207Asn + Tyr208Gly + Asn212Glu
	Gln 57Asn + Asn153Asp + Leu211Ser + Gly213Pro
25	Ala131Asn + Gln185Ser + Val193Ser + Gln200Asn
	Ala181Gly + Ala194Pro + Asn198Ser + Asn212Asp
	Ser101Asp + Leu124Val + Pro195Gln + Thr207Pro
	Gly 95Asp + Asn198Gln + Gln200Ser + Thr207Ser
	Ala 96Gln + Tyr102Pro + Ser182Glu + Val197Asn
30	Asn 60Gln + Gly100Ser + Ser101Asp + Ile159Pro
	Gly 63Asn + Asn153Asp + Gly205Ser + Thr207Ser
	Ser126Glu + Ala131Pro + Asn153Ser + Phe183Thr
	Asn153Gln + Gly205Pro + Ser210Glu + Gly213Pro
	Ile159Glu + Ala194Ser + Pro204Gly + Leu211Ile
35	Asp 58Glu + Val 93Ser + Ala194Pro + Gln200Ser
	Gly 95Asp + Ile105Gln + Phe183Cys + Asn198Gln
	Gly 59Pro + Gly 98Asn + Leu211Val + Thr214Asp
	Gly 61Pro + Val 93Pro + Pro129Asn + Gly213Pro
	Gln 57Asn + Ala156Pro + Ala181Pro + Phe183Gly
40	Gly 95Gln + Gly152Asp + Phe183Met + Asn198Gln
	Gly 59Ser + Gly 95Asn + Pro127Gln + Ser210Glu
	Val197Cys + Asn198Gln + Thr207Glu + Leu211Cys
	Pro129Asn + Ser154Asp + Phe183Pro + Leu211Cys
	Gly 59Gln + Gly100Glu + Ala131Gly + Ala181Gln
45	Gly 61Glu + Tyr161Leu + Pro204Ser + Gly213Pro
	Asp 58Glu + Ile159Met + Tyr161Gln + Gly213Asn
	Ile105Pro + Leu124Asn + Ile159Ser + Asn198Asp
	Tyr102Pro + Ala156Ser + Phe183His + Pro204Asp
	Tyr102Pro + Ile105Pro + Gln200Asn + Pro204Glu
50	Thr 64Ser + Ile105Glu + Gln200Ser + Thr214Pro
	Gly 63Gln + Gly100Asp + Asn198Ser + Leu211His
	Asn 60Asp + Ile105Leu + Gly152Gln + Ala209Gln

Gly152Gln + Gln185Asn + Gly205Ser + Leu211Ala  
 Gln 57Ser + Gly100Pro + Pro127Gly + Asn198Glu  
 Leu 94Cys + Pro127Gly + Val197Gly + Asn198Ser  
 Leu 94Thr + Tyr102Cys + Leu124Cys + Tyr208Ser  
 5 Ala 96Ser + Ala181Asn + Pro204Ser + Tyr208Ile  
 Leu124Val + Asn198Gln + Pro204Gly + Leu211Ile  
 Gly 61Asn + Ala 96Thr + Gly 98Ser + Ser210Asp  
 Thr 64Pro + Ala 96Pro + Gly155Glu + Leu211Cys  
 Gly157Asp + Val197Pro + Asn198Gln + Pro204Gly  
 10 Pro129Gln + Gly155Pro + Val197His + Ser210Glu  
 Val 93Met + Ser182Asp + Gln200Ser + Gly213Ser  
 Leu 94Glu + Pro127Ser + Gln200Asn + Leu211Met  
 Ala 96His + Gly 98Pro + Pro129Ser + Pro204Asp  
 Ser 97Asp + Gly100Pro + Leu124Pro + Thr207Asn  
 15 Gly 59Asn + Thr207Pro + Ser210Glu + Leu211Thr  
 Ala 96Gly + Ala131Asn + Gly155Pro + Ser210Glu  
 Asn153Ser + Val197Thr + Gln200Asn + Ser210Glu  
 Asn 60Ser + Ile105Thr + Ser182Glu + Thr207Ser  
 Gly 98Asn + Tyr161Asn + Gln200Asn + Tyr208Asn  
 20 Leu 94Gly + Ser 99Glu + Leu124Ser + Ala181Pro  
 Ser104Glu + Ala131Pro + Leu211Ser + Gly213Ser  
 Tyr102Asp + Asn198Ser + Thr207Gln + Leu211Asn  
 Gly 98Pro + Ser206Glu + Leu211Gly + Gly213Pro  
 Ala131Asn + Ala181Thr + Tyr208Gln + Ser210Asp  
 25 Gly155Gln + Tyr161Cys + Gln185Ser + Ala209Gln  
 Thr 64Asn + Val 93Asn + Ser126Glu + Gln200Asn  
 Ala131Pro + Phe183Ala + Thr207Asp + Thr214Gln  
 Ser 97Glu + Leu124Pro + Ala131Ser + Pro204Gly  
 Gly 59Asp + Tyr161His + Thr207Gly + Leu211Met  
 30 Gly 95Pro + Gly 98Pro + Ser101Glu + Leu211Cys  
 Gly 59Ser + Thr 64Gly + Leu124Ile + Gly125Glu  
 Thr 64Pro + Ser101Glu + Tyr161Gly + Gln200Asn  
 Gly100Glu + Pro195Ser + Val197Ser + Thr207Asn  
 Ser160Glu + Tyr161Gln + Pro204Ser + Leu211Gln  
 35 Leu124Met + Tyr161Leu + Ser182Glu + Leu211His  
 Ile105Thr + Pro127Asn + Gly157Asn + Tyr161Pro  
 Gln185Asn + Thr207Gln + Asn212Ser + Thr214Gln  
 Val 93Gln + Leu 94Ser + Gly 98Glu + Thr214Gly  
 Asn153Gln + Ser154Glu + Ala181Asn + Leu211Val  
 40 Gly125Asn + Ser154Asp + Val197Ser + Leu211Val  
 Ile159Asn + Gln185Glu + Val197Met + Asn212Gln  
 Ala 96Pro + Gly196Gln + Val197Ala + Asn198Gln  
 Ala131Glu + Gly152Gln + Ala194Ser + Leu211Ile  
 Phe183Ser + Asn198Asp + Gln200Ser + Thr207Pro  
 45 Gly 95Gln + Gly100Ser + Ser130Glu + Thr207Gln  
 Leu124Cys + Gly152Ser + Tyr161Met + Thr207Gln  
 Leu 94Met + Leu124Met + Leu211Ala + Asn212Ser  
 Gly 61Asn + Ala 96Ser + Gly125Pro + Gln200Asp  
 Ile159Gln + Val197Glu + Gln200Asn + Tyr208His  
 50 Tyr161Asn + Ser182Glu + Ala209Ser + Asn212Ser  
 Gln 57Asn + Gly152Ser + Phe183Glu + Gln185Asn  
 Asn198Gln + Thr207Glu + Ala209Gly + Gly213Pro  
 Val197Glu + Tyr208Gly + Ala209Pro + Leu211Pro  
 Ala131His + Gly205Gln + Tyr208Asp + Leu211Gln

5 Gly 61Asn + Val 93Cys + Gly 98Gln + Leu211Glu  
Leu124Ala + Val197Gln + Ser210Glu + Asn212Ser  
Pro127Gln + Ile159Leu + Ser160Glu + Ala209Ser  
Asp 58Glu + Gly 59Gln + Gly205Gln + Leu211Cys  
Asp 58Glu + Phe183Tyr + Asn198Ser + Thr207Gln  
Pro204Gly + Ser206Asp + Thr207Gln + Leu211His  
Gln 57Asp + Gly 98Ser + Ile105Gln + Tyr208Met  
Ser103Glu + Gly155Ser + Ala209His + Leu211Thr  
Ser104Glu + Ala181Asn + Asn198Gln + Leu211Thr  
10 Gln185Asn + Val197Cys + Gly205Asp + Leu211Cys  
Gly 59Gln + Tyr102Glu + Pro127Gly + Ala209Thr  
Ala131Thr + Asn153Ser + Val193Gln + Val197Asp  
Ala131Ser + Asn153Ser + Phe183Tyr + Ala194Ser  
Pro129Gln + Ala181Pro + Ala209His + Leu211Ala  
15 Pro127Gly + Gly196Asn + Ser210Glu + Leu211Met  
Leu 94Ile + Gly100Asp + Asn198Ser + Tyr208Leu  
Asn153Ser + Gly155Pro + Gly205Asp + Leu211Ser  
Gly 63Asn + Gly152Pro + Asn198Gln + Thr207Glu  
Gly152Ser + Ser160Glu + Tyr161His + Leu211Met  
20 Pro129Asn + Asn153Gln + Gly155Gln + Ser210Asp  
Gln 57Ser + Thr 64Gln + Val197Asn + Gln200Asp  
Ser126Asp + Asn153Gln + Pro204Gln + Leu211Gln  
Gly 61Gln + Ala 96Gln + Phe183Leu + Leu211Thr  
Gly 63Asn + Ala156Asn + Asn198Ser + Ser210Glu  
25 Gly 59Asp + Gly205Pro + Thr207Gln + Leu211Asn  
Gly 98Ser + Leu124Pro + Ile159Thr + Val197Glu  
Gly205Pro + Ala209Gln + Ser210Glu + Leu211Pro  
Pro129Ser + Thr207Gly + Ser210Glu + Leu211Asn  
Gln 57Asp + Ala131Gln + Leu211Met + Asn212Gln  
30 Thr 64Asn + Tyr161Ile + Phe183Asn + Ser210Asp  
Gly155Gln + Pro204Ser + Ala209His + Leu211Asp  
Gly100Gln + Gly157Pro + Ile159Leu + Ser160Asp  
Gly 98Pro + Ser103Asp + Ala131Gly + Leu211Asn  
Gln 57Ser + Gly 61Pro + Ile159Gln + Leu211Asn  
35 Gly155Ser + Gln185Asp + Val199Met + Thr207Gly  
Asp 58Glu + Ala 96Gly + Phe183Thr + Thr207Gly  
Val 93Ala + Ser126Glu + Ala131Thr + Thr207Pro  
Thr 64Gln + Gly 98Pro + Val197Thr + Gln200Glu  
Gly152Glu + Asn153Gln + Gly155Pro + Gly213Pro  
40 Ser154Glu + Asn198Ser + Gln200Ser + Leu211Ser  
Ile105Thr + Ile159Thr + Phe183Val + Leu211Asp  
Leu124Pro + Ser128Glu + Ile159Met + Leu211Pro  
Gln 57Asp + Asp 58Glu + Leu211Thr + Thr214Ser  
Gly 59Asp + Asn 60Asp + Val197Pro + Val199Ala  
45 Ala 96Thr + Leu124Cys + Val197Asp + Asn198Glu  
Thr 64Asn + Ala156Gly + Ser210Asp + Leu211Asp  
Leu124Ser + Gln200Asn + Gly205Asp + Ser206Glu  
Gly 63Pro + Ala156Asp + Gly157Asp + Ile159Gln  
Thr 64Gly + Gly100Glu + Ser101Glu + Leu211Asn  
50 Gly 61Asn + Asn153Gln + Gln200Asp + Ser210Asp  
Leu124Ser + Gln200Glu + Ser210Glu + Leu211Gln  
Val197Gln + Gln200Glu + Thr207Ser + Ser210Glu  
Ala209Glu + Ser210Glu + Leu211Glu + Gly213Ser  
Thr207Pro + Ser210Glu + Leu211Asp + Asn212Glu

Ser101Asp + Ser104Asp + Asn198Gln + Leu211Ser  
 Thr 64Asn + Ala 96Asp + Gly 98Asp + Gly213Asn  
 Asn198Glu + Gln200Asn + Ser210Asp + Leu211Thr  
 Tyr161Val + Asn198Glu + Ser210Glu + Gly213Asn  
 Val 93Pro + Asn198Glu + Gln200Ser + Ser210Glu  
 Val193Pro + Asn198Asp + Thr207Ser + Ser210Asp  
 Gln185Ser + Asn198Asp + Gln200Asp + Ser210Glu  
 Gly 95Ser + Gly 98Gln + Asn198Asp + Leu211Asp  
 Val 93His + Ala 96His + Gln200Glu + Ala209Asp  
 Leu 94Cys + Ile159Leu + Gln200Asp + Ala209Glu  
 Gln 57Asn + Gly 61Asp + Thr 64Asp + Leu 94Val  
 Ala156Gln + Asn198Glu + Gly205Gln + Asn212Asp  
 Tyr102Asp + Ser104Asp + Phe183Gln + Val197His  
 Ala 96Ser + Asn153Ser + Gly155Asp + Ser182Asp  
 Ala 96Asn + Asn153Glu + Gln185Glu + Thr214Gly  
 Asp 58Glu + Gly 61Glu + Asn153Ser + Leu211Cys  
 Pro129Ser + Asn198Asp + Gln200Asp + Leu211Pro  
 Gly125Ser + Asn198Asp + Gln200Glu + Leu211Ala  
 Asn198Asp + Gln200Asp + Thr207Pro + Leu211Pro  
 Gly 98Pro + Asn198Asp + Gln200Glu + Tyr203Ser  
 Asn198Glu + Gln200Glu + Tyr203Leu + Thr214Asn  
 Gly100Asp + Tyr102Asp + Asn153Ser + Tyr208Leu  
 Thr 64Gln + Thr207Asn + Ser210Glu + Asn212Glu  
 Pro127Gln + Ile159Cys + Ser210Glu + Asn212Asp  
 Val 93Ala + Ser 99Asp + Ser101Glu + Ile105Asn  
 Gly100Gln + Gly155Gln + Asn212Asp + Thr214Asp  
 Gly 95Ser + Tyr102Leu + Pro129Glu + Ser160Asp  
 Gly 59Ser + Pro129Asp + Ser160Asp + Leu211Pro  
 Thr 64Pro + Ala 96Thr + Asn153Glu + Ser182Asp  
 Asp 58Glu + Gly 61Gln + Gly 95Asp + Ala181Thr  
 Gly 63Pro + Ser 99Asp + Ser101Glu + Ser103Glu  
 Asn153Gln + Val197Asp + Leu211Glu + Thr214Glu  
 Gly152Ser + Ala181Pro + Asn198Asp + Gly213Asp  
 Ser154Glu + Gly155Pro + Ser160Asp + Asn198Gln  
 Gly152Asn + Ser154Glu + Ser158Glu + Leu211Asn  
 Asn 60Glu + Leu 94Asp + Tyr208Ala + Gly213Pro  
 Thr 64Asp + Gln200Asp + Ser210Asp + Gly213Pro  
 Ser103Asp + Ser130Glu + Ile159Ala + Thr202Gln  
 Ser103Glu + Ser130Glu + Asn198Gln + Pro204Asn  
 Thr207Pro + Ser210Asp + Leu211Glu + Thr214Glu  
 Ser210Glu + Leu211Met + Gly213Glu + Thr214Glu  
 Gly125Ser + Gln200Asp + Ser206Glu + Thr207Glu  
 Ala 96Pro + Gly125Asp + Ser154Asp + Ala156Asp  
 Ser 99Asp + Ser104Asp + Ile105Met + Gln200Ser  
 Gly 59Gln + Ser128Asp + Ser154Glu + Ser160Asp  
 Asp 58Glu + Gly 98Asp + Leu124Ser + Tyr161Met  
 Gln185Glu + Asn198Ser + Leu211Cys + Gly213Asp  
 Gly 61Gln + Gly 98Pro + Gln200Asp + Thr207Glu  
 Gly100Gln + Gln200Glu + Tyr203Pro + Thr207Glu  
 Val197Pro + Gln200Asp + Thr207Asp + Ala209Gln  
 Asp 58Glu + Val 93Gly + Ser 97Glu + Tyr161Ile  
 Asp 58Glu + Ser 97Asp + Gln200Ser + Tyr208Thr  
 Gly152Asp + Gly157Glu + Val199Ser + Thr207Ser  
 Val197Gln + Pro204Ser + Thr207Asp + Ser210Asp

5 Gly125Asn + Prol29Asn + Thr207Glu + Ser210Glu  
Ser101Asp + Ser128Asp + Prol29Glu + Gly152Ser  
Leu 94Ala + Asn198Glu + Gln200Asp + Thr214Glu  
Ala 96Ser + Ile105Glu + Ser126Glu + Ser160Asp  
Ser130Asp + Ser158Glu + Ser160Asp + Asn198Gln  
Leu124Asp + Phe183Glu + Tyr208Val + Gly213Asp  
Ser101Asp + Ser126Glu + Ala156Gly + Thr207Asn  
Asp 58Glu + Asn 60Asp + Gly100Asp + Ala181Gln  
Asp 58Glu + Gly205Ser + Thr207Asp + Tyr208Glu  
10 Thr 64Gln + Leu 94Glu + Ser 97Asp + Gly125Asp  
Ser158Glu + Ile159Asp + Leu211Val + Gly213Glu  
Gln 57Asp + Thr 64Asp + Leu124Thr + Gln200Asp  
Gly 61Glu + Gln200Ser + Gly205Glu + Ser210Glu  
Asp 58Glu + Ile105Gly + Leu211Asp + Asn212Glu  
15 Gln 57Asp + Asp 58Glu + Gln200Glu + Pro204Ser  
Gln 57Asp + Asp 58Glu + Ala 96His + Ser160Glu  
Gly157Asp + Gln200Ser + Ala209Glu + Ser210Glu  
Gly 59Glu + Asn 60Asp + Gly 95Asn + Gln185Glu  
Ser101Asp + Tyr102His + Val197Glu + Asn198Glu  
20 Gly152Asp + Asn153Glu + Ser210Asp + Leu211Met  
Gly 59Asp + Tyr102Ile + Ser210Asp + Leu211Asp  
Ser101Glu + Tyr102Asp + Gln200Glu + Leu211Asn  
Gly 59Asn + Ser130Glu + Gly205Asp + Ser206Asp  
Ser128Asp + Tyr161Gly + Ser206Asp + Thr207Glu  
25 Asn 60Gln + Ser 97Glu + Ser182Asp + Phe183Asp  
Gly 98Glu + Ser 99Asp + Ser160Glu + Thr207Gln  
Gly 98Asp + Thr202Gln + Thr207Glu + Tyr208Asp  
Ser154Glu + Tyr161Leu + Ser210Glu + Asn212Glu  
Asn 60Asp + Ile105Ser + Gln200Asp + Ser210Glu  
30 Ala131Asp + Gln200Glu + Ser210Asp + Leu211Pro  
Gln 57Asp + Gln200Glu + Ser210Asp + Gly213Pro  
Prol29Gln + Ser160Glu + Gln200Asp + Ser210Glu  
Prol29Asp + Asn198Gln + Gln200Asp + Ser210Glu  
Gly 59Glu + Thr 64Gly + Gln200Glu + Ser210Glu  
35 Asn 60Glu + Ala 96Glu + Asn198Gln + Ser210Glu  
Asn 60Asp + Ala 96Asp + Ser130Glu + Ala156Pro  
Gln 57Ser + Ile159Ser + Gln200Asp + Gly213Glu  
Gly155Asp + Gln185Glu + Asn198Glu + Tyr203His  
Ser104Glu + Leu124Asp + Gln185Ser + Val197Gly  
40 Gly 59Gln + Ser101Glu + Ser103Asp + Asn198Asp  
Asn 60Gln + Ser126Asp + Ser128Asp + Ser210Glu  
Ser126Glu + Ser128Asp + Gly155Asn + Asn212Glu  
Ser103Asp + Asn198Glu + Ser210Glu + Thr214Gln  
Ser160Glu + Asn198Glu + Ser210Asp + Leu211Ser  
45 Asp 58Glu + Asn198Asp + Thr207Pro + Ser210Asp  
Gly 98Asn + Ser103Glu + Asn198Glu + Ser210Glu  
Ser128Asp + Gly155Pro + Asn198Asp + Ser210Asp  
Gly152Glu + Asn198Asp + Thr207Asn + Ser210Asp  
Ser101Asp + Prol29Gly + Asn198Glu + Leu211Glu  
50 Asn198Glu + Tyr208Asp + Gly213Ser + Thr214Ser  
Ser 97Glu + Gly152Glu + Ser154Asp + Gln185Ser  
Thr 64Asp + Ser210Asp + Leu211Val + Gly213Gln  
Thr 64Glu + Gly205Ser + Ser210Asp + Leu211Asn  
Leu 94Thr + Ser126Glu + Pro204Glu + Thr207Asp



Gly100Gln + Tyr102Asp + Pro204Asp + Thr207Asp  
 Ser101Glu + Asn198Glu + Gln200Glu + Leu211Ala  
 Thr 64Asp + Ser 99Glu + Pro204Glu + Thr207Asn  
 Gly155Pro + Ser182Asp + Gln200Asp + Leu211Glu  
 5 Gly100Asn + Ser160Glu + Gln200Glu + Leu211Glu  
 Asp 58Glu + Val 93Asn + Ser206Glu + Leu211Met  
 Ala131Pro + Asn153Glu + Ser182Asp + Asn198Glu  
 Asp 58Glu + Ala 96Glu + Pro127Ser + Gly157Glu  
 10 Leu 94Asp + Tyr102Leu + Thr207Glu + Ala209Glu  
 Ala 96Gln + Ser158Asp + Ser160Glu + Val197Glu  
 Gly100Asp + Ser126Glu + Ser154Asp + Ile159Gly  
 Tyr102Asn + Gln200Glu + Thr207Glu + Gly213Asp  
 Val 93Glu + Pro127Ser + Ser210Asp + Asn212Asp  
 Ile105Asp + Ala131Glu + Ala156Gln + Thr202Pro  
 15 Gln 57Ser + Ser126Glu + Gly152Asp + Ser210Glu  
 Ser126Glu + Gly152Glu + Asn198Glu + Thr207Asn  
 Leu 94Glu + Ser101Asp + Tyr102Leu + Thr207Glu  
 Leu 94Asp + Ser101Asp + Ser154Asp + Gly213Asn  
 Asn 60Asp + Thr 64Glu + Ser103Glu + Thr214Asn  
 20 Asp 58Glu + Val197Asp + Ser210Glu + Leu211Pro  
 Ser160Asp + Phe183Tyr + Val197Asp + Ser210Asp  
 Pro129Glu + Val197Glu + Ser210Glu + Leu211His  
 Ser126Asp + Ser130Glu + Ser182Glu + Gln200Ser  
 Ser126Asp + Ser130Glu + Asn198Asp + Leu211His  
 25 Thr 64Ser + Pro204Asp + Ser210Asp + Leu211Ile  
 Ser 97Asp + Gly100Glu + Gly152Pro + Asn198Glu  
 Ser 97Asp + Gly100Glu + Gln200Glu + Tyr208Gly  
 Asp 58Glu + Gly 59Pro + Ser154Asp + Gly157Asp  
 Gly155Gln + Ala181Glu + Gln185Asp + Ser210Asp  
 30 Gly125Glu + Asn153Asp + Gln200Asp + Tyr208Pro  
 Gln 57Ser + Ser101Asp + Ser154Asp + Ser160Asp  
 Ser104Asp + Asn198Glu + Ala209Asp + Leu211Met  
 Leu124Ile + Gly125Pro + Pro129Glu + Ser158Glu  
 Ser154Glu + Ser158Asp + Gly205Pro + Thr207Glu  
 35 Gly152Glu + Ser158Asp + Gln200Asp + Thr207Pro  
 Ala131Asp + Gly152Asp + Ser158Asp + Val197Thr  
 Ser103Glu + Gln200Glu + Thr207Pro + Asn212Asp  
 Ser130Glu + Gln200Glu + Thr202Ser + Asn212Glu  
 Asn 60Glu + Leu 94Glu + Ile105Leu + Ser130Glu  
 40 Ser 99Glu + Pro127Asp + Pro129Ser + Ser154Glu  
 Asn153Glu + Ile159Gly + Leu211Glu + Gly213Asn  
 Ala156Asp + Ser160Glu + Gly205Gln + Leu211Asp  
 Ser104Asp + Ser130Asp + Gly155Pro + Gly157Asn  
 Gly 98Asp + Ser101Asp + Ser128Asp + Ala181Gln  
 45 Asn 60Asp + Tyr102Gly + Gln200Glu + Thr207Asp  
 Gly 59Pro + Ala131Asp + Ser154Asp + Thr214Asp  
 Ser103Glu + Ser130Glu + Asn198Glu + Asn212Gln  
 Asn 60Asp + Gly 98Asn + Ser103Glu + Ser130Asp  
 Ser160Asp + Phe183Ser + Ser210Glu + Thr214Asp  
 50 Asp 58Glu + Ser 99Asp + Val197Pro + Pro204Asn  
 Asp 58Glu + Gly 98Glu + Gly155Gln + Leu211Asp  
 Thr 64Glu + Leu 94Asp + Gln185Ser + Ser210Asp  
 Asn 60Asp + Ser 99Glu + Ala181Glu + Val197Asn  
 Ser128Glu + Ser206Asp + Ala209Glu + Asn212Gln

	Gly100Asp + Gln185Glu + Leu211Ser + Gly213Glu
	Ser126Glu + Ser154Glu + Asn198Gln + Gly205Glu
	Gly125Glu + Val197Thr + Gln200Glu + Thr207Asp
5	Asn 60Glu + Ile105Pro + Ile159Val + Leu211Glu
	Pro129Asn + Gln200Glu + Pro204Asp + Thr207Gln
	Asn198Ser + Gln200Asp + Tyr203Ile + Pro204Glu
	Gly 98Ser + Ser103Glu + Ala131Glu + Ser210Asp
	Ser103Glu + Ala131Asp + Tyr161His + Ser210Glu
10	Gly 61Glu + Gly125Pro + Ser126Glu + Thr207Glu
	Gly152Asp + Gly157Asp + Pro204Asp + Ala209Thr
	Pro127Asp + Asn153Glu + Pro204Ser + Ser206Asp
	Gln 57Asp + Gln200Ser + Thr207Glu + Ser210Glu
	Ser154Asp + Thr207Glu + Ser210Glu + Thr214Gln
15	Ser130Glu + Pro204Asn + Thr207Glu + Ser210Asp
	Asp 58Glu + Val 93Ala + Ser101Glu + Ser130Asp
	Gly125Glu + Ser130Asp + Gln200Asp + Leu211Thr
	Leu 94Glu + Ser182Asp + Thr207Gln + Asn212Glu
	Ser 97Glu + Ile159Asp + Phe183Ile + Thr214Asp
	Gly 95Glu + Ser103Asp + Pro129Asn + Ala131Pro
20	Asn 60Gln + Gly 61Glu + Ser130Asp + Ser210Asp
	Ser101Glu + Ser126Asp + Ala209Glu + Asn212Ser
	Ser101Glu + Ser126Asp + Tyr161Leu + Gly205Glu
	Asn 60Ser + Gly 98Asp + Ser126Glu + Leu211Thr
25	Val 93Glu + Ser 97Glu + Pro129Ser + Gln200Glu
	Thr 64Asn + Gln200Asn + Thr207Asp + Leu211Asp
	Gly 98Glu + Ser104Asp + Ser126Asp + Phe183Gly
	Gly125Asp + Gly157Asp + Thr207Gln + Gly213Glu
	Leu 94Asp + Ser128Asp + Gly152Asp + Leu211Met
30	Ser158Glu + Ser182Glu + Thr207Gly + Tyr208Cys
	Gln200Asn + Pro204Gln + Ser210Glu + Thr214Glu
	Thr 64Glu + Ala131Asp + Ser210Asp + Leu211Ala
	Asp 58Glu + Gly 59Pro + Gly100Pro + Thr207Asp
	Asn198Gln + Gln200Glu + Ser206Glu + Tyr208His
35	Gly 95Pro + Ser126Asp + Ala131Glu + Gln200Glu
	Ser 97Glu + Ser101Glu + Gln200Asp + Pro204Asn
	Ser 97Asp + Ser101Asp + Gly125Gln + Ser210Asp
	Ala131Gly + Ser154Asp + Tyr161Glu + Ser210Asp
	Ser126Glu + Pro204Asp + Thr207Gly + Ser210Asp
40	Gly 98Glu + Ser104Asp + Val197Glu + Asn198Ser
	Ser104Asp + Ser130Glu + Ala156Thr + Thr207Asp
	Ser104Asp + Ser130Asp + Ala131Thr + Thr207Asp
	Pro127Ser + Ser158Asp + Gln200Ser + Thr214Glu
	Asn 60Asp + Tyr102Ser + Val197Asn + Thr207Asp
45	Ser101Asp + Pro127Ser + Ala131Glu + Ala156Glu
	Asp 58Glu + Ser 99Asp + Gln200Glu + Tyr208Ser
	Asp 58Glu + Ser 99Asp + Leu124His + Asn198Glu
	Gly 61Glu + Thr 64Ser + Val 93Asp + Tyr208Gly
	Asn 60Glu + Gln185Ser + Asn198Ser + Ser206Asp
50	Gly 61Pro + Ser158Asp + Ala181Glu + Leu211Asp
	Tyr161Glu + Gln200Glu + Tyr203His + Pro204Glu
	Gly 61Glu + Gly157Asp + Leu211Met + Asn212Asp
	Gly 59Ser + Gly 98Glu + Tyr102Glu + Ser158Asp

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TABLE 36

Multi-loop Quintuple Mutation Variants					
5	Val 93Gln + Tyr102Cys + Ser154Glu + Asn198Gln + Thr207Gly				
	Leu 94Gly + Leu124Ile + Asn198Gln + Ala209Gly + Ser210Glu				
	Leu124Asp + Ala156His + Gln200Ser + Thr207Pro + Leu211Thr				
	Pro129Asn + Gly155Asp + Gly157Asn + Thr207Ser + Asn212Gln				
	Gly 61Ser + Gly 98Glu + Pro127Asn + Gln200Ser + Thr207Pro				
10	Ala156Gln + Gln185Ser + Gln200Asn + Ser210Asp + Leu211His				
	Gly 63Asn + Tyr102Pro + Ala156Gln + Ser182Glu + Val197Asn				
	Ala 96Pro + Ile105Pro + Thr207Ser + Leu211Ile + Thr214Pro				
	Val 93Asn + Val197Gly + Asn198Gln + Ser210Asp + Leu211Ser				
	Val 93Pro + Leu 94Ile + Ile159Asn + Gln200Ser + Thr207Ser				
15	Gly 61Pro + Gly 95Pro + Ser154Glu + Ala181His + Gly213Pro				
	Gly157Ser + Ser182Glu + Pro195Gln + Thr207Pro + Leu211Ser				
	Ser 97Glu + Tyr102Met + Asn198Ser + Ala209Gln + Leu211Ala				
	Leu124His + Ala131His + Gly157Pro + Asn198Asp + Thr202Ser				
	Gly 95Asp + Ala181Pro + Gln200Asn + Leu211Ala + Gly213Gln				
20	Leu 94Ile + Gly 95Asn + Ser158Glu + Leu211Met + Gly213Ser				
	Gly 98Asn + Gly125Pro + Pro127Gly + Tyr161Ile + Tyr208Met				
	Ala 96Ser + Ser154Glu + Tyr161Gly + Gly196Gln + Val197Gln				
	Leu 94Ser + Gly 95Asn + Pro127Asn + Val197Ser + Thr214Asn				
	Asp 58Glu + Val 93Gln + Leu 94Met + Gln200Asn + Thr207Gln				
25	Gly 95Asp + Gly155Ser + Val197Thr + Thr207Gly + Leu211Pro				
	Gly 98Ser + Ala131Glu + Val197Gln + Gln200Ser + Pro204Gly				
	Gln 57Asn + Val 93Thr + Tyr102Ser + Leu124Pro + Ser126Asp				
	Ser101Asp + Tyr102Cys + Leu124Val + Phe183Gly + Val199Asn				
	Asp 58Glu + Thr 64Gln + Ile105Pro + Gly125Gln + Asn198Ser				
30	Gly155Glu + Tyr161Val + Pro204Gly + Thr207Pro + Leu211Ile				
	Gly100Gln + Ser182Asp + Phe183Asn + Asn198Ser + Thr207Gln				
	Gly 98Glu + Pro129Gly + Gly155Pro + Thr207Gly + Thr214Gly				
	Leu 94Met + Gly100Ser + Gly196Gln + Gln200Asn + Asn212Asp				
	Ser104Glu + Gly152Gln + Asn153Gln + Ala194Gln + Asn212Ser				
35	Pro129Gln + Val197Thr + Tyr208Ile + Ser210Glu + Thr214Ser				
	Val 93Gly + Ala 96Asn + Pro129Gln + Ser160Asp + Pro204Gly				
	Pro127Gln + Ala131Asn + Gly196Ser + Val197Thr + Ser206Glu				
	Pro127Ser + Ser128Asp + Ala156His + Ala194Gln + Leu211Ala				
	Gly 95Gln + Pro127Gly + Val197Gly + Gly205Asn + Ser210Glu				
40	Ala131His + Pro204Gly + Thr207Asn + Leu211Thr + Gly213Asn				
	Leu 94Gly + Tyr102Gln + Phe183Val + Thr207Ser + Leu211Asp				
	Gly 61Asn + Ile105Met + Gly125Ser + Val193Gly + Asn198Glu				
	Gly 59Pro + Ala 96Gly + Ser101Asp + Tyr208His + Leu211His				
	Tyr102Val + Gln200Asp + Pro204Ser + Leu211Ile + Thr214Pro				
45	Val 93Thr + Ser104Glu + Pro195Ser + Val199Pro + Leu211Val				
	Ile159Gln + Tyr161Ala + Phe183Leu + Ser210Glu + Thr214Gly				
	Gly 59Asn + Pro127Ser + Ile159Ala + Gln200Asn + Leu211Met				
	Gln 57Glu + Val 93His + Gly125Gln + Ala131Thr + Ala156Asn				
	Ser126Asp + Gly157Ser + Asn198Gln + Gln200Ser + Thr207Ser				
50	Gly125Asn + Pro129Ser + Phe183Asp + Thr207Gly + Leu211Gly				
	Asn 60Asp + Leu 94Met + Tyr102Pro + Gly152Asn + Thr207Gln				
	Ile105Leu + Gly152Pro + Ser158Glu + Asn198Ser + Thr214Gly				
	Ala 96Asn + Ala131Thr + Gly157Asn + Thr207Gly + Ser210Glu				
	Val 93Gln + Leu124Met + Ala181Gln + Asn198Glu + Gln200Ser				
	Pro127Ser + Ile159Thr + Pro195Gly + Asn198Ser + Gln200Glu				

Ala 96Asn + Val197His + Asn198Ser + Gln200Asn + Asn212Asp  
 Asp 58Glu + Leu 94Ala + Leu124Pro + Ala156Gly + Leu211Gln  
 Ile105Thr + Gly157Pro + Phe183Pro + Val197Asp + Tyr208Val  
 Gly 95Glu + Gly100Gln + Tyr102Gly + Tyr161His + Tyr208Cys  
 5 Thr 64Gly + Val 93Asp + Gly 95Pro + Gly100Pro + Asn198Gln  
 Gln 57Asn + Asn 60Gln + Ser101Glu + Gly155Ser + Pro204Ser  
 Gly125Ser + Gln185Asn + Asn198Gln + Ser206Glu + Ala209His  
 Gly 98Ser + Pro129Asn + Val197Glu + Gln200Asn + Tyr208Asn  
 Gly 98Asp + Asn153Gln + Gln200Asn + Pro204Ser + Leu211Ser  
 10 Ala 96Thr + Ala181Thr + Val197Asn + Gln200Asn + Thr207Pro  
 Gly 95Gln + Pro129Asn + Tyr161Ala + Gln185Asp + Thr207Asn  
 Gly 95Asn + Gly152Gln + Ser160Glu + Ala194Asn + Tyr208His  
 Gln 57Asp + Phe183Leu + Thr207Ser + Ala209Gln + Leu211Met  
 Ile105His + Gly125Asp + Gly152Asn + Pro204Asn + Tyr208Leu  
 15 Gln 57Asn + Leu 94Glu + Ala 96Gln + Pro127Gly + Leu211Pro  
 Asn153Ser + Tyr161Met + Ala181Gln + Asn198Ser + Ser210Asp  
 Gly 95Asn + Gly 98Glu + Pro129Asn + Ala209Pro + Thr214Pro  
 Gly 61Asn + Asn198Ser + Ala209Glu + Leu211Ser + Gly213Pro  
 Ser 99Asp + Pro127Asn + Pro195Asn + Tyr208His + Leu211Cys  
 20 Asn 60Gln + Thr 64Asn + Gly100Asn + Ile105Leu + Pro127Asn  
 Pro127Glu + Ala194Gln + Gln200Asn + Thr207Ser + Leu211Cys  
 Thr 64Gly + Leu 94Gly + Val197Asn + Gln200Ser + Ala209Pro  
 Gly 59Gln + Leu 94Ser + Ala 96Ser + Leu211Thr + Thr214Glu  
 Pro129Gly + Ala194Asn + Gln200Ser + Leu211Gln + Asn212Asp  
 25 Ala131Gly + Asn153Glu + Thr207Asn + Leu211Thr + Gly213Gln  
 Gly 59Pro + Thr 64Asn + Asn198Gln + Ala209Glu + Ser210Asp  
 Gly155Asn + Ser160Asp + Tyr161Glu + Asn198Ser + Leu211Ser  
 Ser126Glu + Pro127Glu + Gln200Ser + Thr207Ser + Leu211Pro  
 Ala131Gly + Ala209Thr + Ser210Asp + Leu211Glu + Thr214Gly  
 30 Gly 61Asn + Ile159Ser + Ser206Glu + Thr207Asp + Leu211Ala  
 Gly 98Glu + Ser 99Glu + Tyr161Leu + Phe183Gln + Asn198Gln  
 Gln 57Ser + Val 93Asn + Gly155Asn + Gln200Glu + Ser210Glu  
 Thr 64Asn + Gly 95Glu + Ala 96Asp + Gly 98Asp + Gln200Ser  
 Ile105Ser + Ser210Glu + Leu211Asp + Asn212Asp + Gly213Ser  
 35 Ala 96Asn + Gly100Ser + Ile105Cys + Asn198Asp + Ser210Glu  
 Ala 96Gln + Gly 98Asn + Asn198Asp + Pro204Asn + Ser210Asp  
 Gly 98Gln + Asn198Glu + Gln200Glu + Tyr208Asn + Ser210Asp  
 Ile105Pro + Leu124Asn + Ile159Ser + Asn198Asp + Leu211Asp  
 Leu 94Gln + Tyr161Pro + Asn198Glu + Thr207Gly + Leu211Glu  
 40 Tyr161Gly + Val197Ala + Asn198Gln + Pro204Glu + Ser206Asp  
 Leu124Ala + Asn198Asp + Tyr208Gly + Ser210Glu + Asn212Asp  
 Gln185Asn + Asn198Asp + Gln200Glu + Thr207Gln + Leu211Asp  
 Pro127Gly + Tyr161Ala + Pro204Glu + Thr207Asp + Thr214Ser  
 Gln 57Asn + Leu124Ser + Val197Glu + Asn198Glu + Gln200Glu  
 45 Asp 58Glu + Ala 96Asp + Val197Ala + Gly205Ser + Thr207Gly  
 Ser158Asp + Ser160Asp + Pro195Ser + Thr207Ser + Leu211Met  
 Asp 58Glu + Asn 60Glu + Thr 64Glu + Gly157Asn + Leu211Ala  
 Leu124Glu + Ser126Asp + Ile159Met + Phe183Val + Val193His  
 Leu 94Ile + Pro127Glu + Pro129Asp + Ser130Asp + Tyr203His  
 50 Leu 94Cys + Ser 99Asp + Ser101Glu + Asn198Gln + Asn212Gln  
 Gly 61Asn + Ser128Glu + Ser160Glu + Ala181Asn + Thr207Gln  
 Tyr161Met + Val197Asp + Gln200Asp + Ser210Glu + Asn212Gln  
 Val 93Ala + Gly125Pro + Ser154Glu + Gly157Asp + Ser158Asp  
 Leu 94Glu + Ser101Asp + Phe183Ala + Gln200Asn + Leu211Met

Leu 94Asp + Ser103Glu + Ser104Asp + Asn198Ser + Tyr208Gly  
 Gly125Asp + Asn153Glu + Ser160Glu + Tyr161Asn + Asn198Ser  
 Leu 94Asn + Pro127Asn + Ser154Asp + Gln185Glu + Thr214Asp  
 Gly 61Gln + Thr 64Asn + Asn198Glu + Ser210Asp + Gly213Asp  
 5 Leu124Gln + Asn198Glu + Gln200Asp + Thr207Gly + Tyr208Glu  
 Leu 94Glu + Leu124Asp + Pro129Asn + Ala156Ser + Thr207Gly  
 Gly 98Asn + Ala156Pro + Asn198Glu + Gln200Asn + Gly213Asp  
 Gly 95Glu + Gly 98Gln + Ser 99Glu + Tyr102Cys + Ser104Glu  
 Gly125Asn + Gln200Asp + Thr207Glu + Ser210Asp + Leu211His  
 10 Gly 59Asp + Ser 97Asp + Ser 99Asp + Ala131Pro + Pro204Gly  
 Gln 57Ser + Tyr102Gln + Asn198Glu + Ala209Glu + Leu211Ser  
 Asn153Asp + Ile159Asp + Tyr161His + Phe183His + Gly213Pro  
 Gly100Ser + Gly155Asp + Ile159Glu + Val197Met + Thr207Pro  
 Pro127Ser + Gln200Asp + Thr207Gly + Ser210Asp + Gly213Glu  
 15 Ala 96Thr + Ser126Glu + Gly152Glu + Ser158Glu + Thr207Asn  
 Leu 94Ala + Gly 95Asp + Ser 97Asp + Ser101Asp + Thr202Gly  
 Gly152Ser + Gly205Ser + Thr207Glu + Ser210Glu + Leu211Asp  
 Tyr161Pro + Pro195Ser + Thr207Asp + Ser210Glu + Leu211Asp  
 Asp 58Glu + Ser 97Glu + Ser 99Asp + Gln185Asn + Gly196Ser  
 20 Thr 64Gly + Ser101Glu + Ile105Glu + Ser126Asp + Tyr161Leu  
 Thr 64Gly + Ser 99Asp + Ile105Glu + Gly205Pro + Leu211Ala  
 Asn153Glu + Ala194Thr + Asn198Glu + Tyr208Ile + Gly213Glu  
 Gly152Gln + Gln200Asn + Ser206Glu + Ala209Asp + Ser210Glu  
 Ser103Glu + Gly125Pro + Ala131Glu + Tyr161Cys + Leu211Thr  
 25 Gly100Ser + Gln200Asn + Thr207Glu + Ser210Asp + Leu211Gln  
 Gly100Pro + Asn198Asp + Gln200Glu + Gly205Gln + Thr207Glu  
 Ala 96Asp + Ser 99Glu + Ser104Asp + Val197Met + Gly213Ser  
 Gly 98Asp + Gly100Pro + Ser101Asp + Gly125Ser + Ala156Ser  
 Gly 98Asp + Gly125Asp + Ser160Glu + Tyr161Ile + Thr214Gly  
 30 Gly 61Glu + Gly155Gln + Asn198Gln + Ser206Glu + Tyr208His  
 Asp 58Glu + Asn 60Glu + Leu124Asn + Ser206Asp + Thr207Asn  
 Gly 61Asp + Thr202Pro + Thr207Gln + Ser210Glu + Leu211Pro  
 Gly 61Glu + Tyr102His + Phe183His + Gln185Asn + Ser210Asp  
 Ser 99Asp + Ser126Asp + Pro129Asp + Gly152Ser + Leu211His  
 35 Leu 94Ile + Gly125Gln + Ser128Asp + Pro129Glu + Ser154Asp  
 Asn198Asp + Gln200Ser + Gly205Pro + Gly213Ser + Thr214Asp  
 Gly 61Asn + Ser182Asp + Val193Gln + Asn198Asp + Ser210Glu  
 Gly 61Glu + Leu 94Cys + Gln200Ser + Gly205Glu + Ser210Glu  
 Asp 58Glu + Phe183Ser + Pro204Glu + Thr207Ser + Ser210Glu  
 40 Leu 94Met + Ala156Asp + Tyr161Cys + Asn212Glu + Thr214Glu  
 Asp 58Glu + Leu124Pro + Ile159Thr + Leu211Asp + Asn212Glu  
 Gln 57Glu + Leu 94Thr + Val197Ser + Ala209Glu + Ser210Asp  
 Ser104Glu + Pro129Ser + Ala156Gly + Ala209Glu + Ser210Glu  
 Asn153Asp + Ser154Glu + Asn198Asp + Thr207Gln + Leu211Gln  
 45 Gly 59Asp + Asn 60Glu + Ser101Glu + Tyr102His + Thr207Asn  
 Gly 61Gln + Ser104Asp + Ile105Asp + Asn153Ser + Thr207Asp  
 Ser 97Glu + Val197Gln + Asn198Gln + Asn212Asp + Gly213Asp  
 Tyr102Val + Ser154Glu + Gly205Glu + Ser206Asp + Leu211Cys  
 Asn153Glu + Tyr161Thr + Pro195Gly + Ser206Asp + Thr207Glu  
 50 Gly 59Gln + Thr 64Pro + Gly 98Glu + Ser130Asp + Ala131Glu  
 Gly125Pro + Gly152Asn + Ser158Glu + Ile159Glu + Ser210Glu  
 Ser 97Asp + Gly 98Asp + Ile105Val + Leu124Thr + Gln200Asp  
 Gly100Glu + Ser101Asp + Ser160Asp + Val197Ala + Leu211Gln  
 Ser103Asp + Ser104Asp + Ala181Ser + Asn198Asp + Tyr208Ile

Ser 99Glu + Gly100Glu + Gly125Asn + Ser182Asp + Thr207Gly  
 Leu 94Gly + Gly100Asp + Ala131Gly + Gln200Glu + Ser210Asp  
 Ser 97Glu + Ala131Gln + Gln200Asp + Thr207Gly + Ser210Glu  
 Gly125Asn + Ser130Glu + Phe183Asn + Gln200Asp + Ser210Glu  
 5 Gly 95Gln + Tyr102Leu + Pro127Glu + Gln200Asp + Ser210Asp  
 Gly125Glu + Gly155Asn + Gln200Glu + Ser210Asp + Thr214Gly  
 Gln 57Ser + Asp 58Glu + Gln200Asp + Thr207Gly + Ser210Glu  
 Pro127Asp + Tyr161Cys + Gln185Asn + Gln200Asp + Ser210Asp  
 Gly100Glu + Gln200Asp + Thr207Pro + Ser210Asp + Leu211Ala  
 10 Gly 59Pro + Ser160Asp + Asn198Gln + Gln200Asp + Ser210Glu  
 Thr 64Glu + Ile105Cys + Leu124Ser + Val197Glu + Ser210Asp  
 Gln 57Glu + Val 93Glu + Gly 95Ser + Ser103Glu + Asn198Gln  
 Asn 60Glu + Ala 96Asp + Tyr102Glu + Gln200Ser + Thr207Gly  
 Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu + Gly213Asp  
 15 Asn198Asp + Pro204Gln + Ser206Glu + Ser210Glu + Leu211Thr  
 Val 93Glu + Ser126Glu + Ser160Asp + Val197Gln + Leu211Ser  
 Gly 98Asp + Ser126Glu + Pro129Asp + Ala181Ser + Gly213Ser  
 Ala 96Glu + Gly 98Glu + Ser103Asp + Pro127Ser + Ile159Pro  
 Gly 63Gln + Ser128Glu + Gly155Ser + Asn198Glu + Ser210Glu  
 20 Gln 57Asp + Ala 96Thr + Gln185Asn + Asn198Asp + Ser210Asp  
 Asn 60Gln + Gly 95Asp + Asn198Glu + Gln200Asn + Ser210Asp  
 Ser104Asp + Ile105Met + Asn198Asp + Ser210Asp + Leu211Asn  
 Tyr102Glu + Asn198Asp + Gln200Asn + Ser210Asp + Leu211Val  
 Gln 57Asp + Gly125Ser + Asn198Asp + Gly205Asn + Ser210Asp  
 25 Tyr102Leu + Gln185Glu + Asn198Glu + Gln200Ser + Ser210Glu  
 Gly 59Asp + Tyr102Ile + Asn198Glu + Ser210Asp + Thr214Ser  
 Val 93Thr + Leu124Val + Ile159Glu + Asn198Asp + Ser210Glu  
 Ser101Asp + Pro129Gly + Asn198Glu + Thr207Ser + Leu211Glu  
 Gly152Gln + Gly155Gln + Asn198Asp + Gly205Asp + Leu211Asp  
 30 Gly 59Gln + Thr 64Ser + Ala156Asp + Ser158Glu + Gly205Glu  
 Pro127Glu + Asn198Ser + Pro204Glu + Ser206Asp + Thr207Pro  
 Asp 58Glu + Ile159Gly + Asn198Asp + Leu211Ile + Asn212Glu  
 Ser130Asp + Ile159Gly + Asn198Glu + Leu211Met + Asn212Glu  
 Asp 58Glu + Asn 60Glu + Ile159Met + Tyr161Gln + Gln200Asp  
 35 Ser 97Asp + Ser103Glu + Ile105Glu + Val197Cys + Leu211Val  
 Ser103Glu + Ile105Glu + Asn153Ser + Gln200Ser + Ser210Asp  
 Ala 96Pro + Gly152Asp + Ser160Asp + Thr207Glu + Leu211Gln  
 Gly 95Asp + Ser 99Glu + Ile105Gly + Gly157Pro + Leu211Glu  
 Ser 97Asp + Ser 99Glu + Val197Glu + Asn198Ser + Thr214Gly  
 40 Gln 57Asn + Ser 97Glu + Ser 99Glu + Asn153Glu + Val193His  
 Thr 64Glu + Ala131Asn + Tyr208Leu + Ser210Asp + Gly213Ser  
 Gln 57Glu + Ala156Pro + Ser158Asp + Gln185Glu + Thr207Gln  
 Ser 97Asp + Pro129Asn + Ser158Glu + Gln185Glu + Leu211Ser  
 Ala 96Thr + Ala156His + Ser158Glu + Gln185Asp + Leu211Asp  
 45 Leu 94Ala + Ser101Asp + Ser126Glu + Ala131Gln + Ser154Asp  
 Gly157Glu + Ile159Leu + Gln200Asn + Pro204Asp + Thr207Glu  
 Leu 94Thr + Ser126Glu + Pro204Glu + Thr207Asp + Gly213Asn  
 Gly100Gln + Ser154Glu + Asn198Glu + Gln200Asp + Leu211Ser  
 Gln 57Glu + Asn198Glu + Gln200Glu + Tyr203Val + Gly213Pro  
 50 Ile105Met + Ser158Glu + Ile159Ser + Asn198Glu + Gln200Asp  
 Ser104Asp + Ala181Gln + Asn198Glu + Gln200Asp + Thr207Gly  
 Gly100Asn + Gly152Pro + Ser182Asp + Gln200Asp + Gly213Asp  
 Tyr102Met + Ser128Glu + Ser130Asp + Asn198Asp + Leu211Met  
 Ser128Glu + Ser130Glu + Ala131Thr + Asn198Glu + Asn212Gln

Val 93Thr + Leu124Glu + Ile159Asn + Gln200Glu + Leu211Asp  
 Gly157Ser + Ser182Asp + Gln200Glu + Thr207Pro + Leu211Glu  
 Ser130Glu + Gln200Asp + Gly205Gln + Thr207Gln + Leu211Glu  
 Gln 57Asn + Gly 59Asn + Asn 60Glu + Gly 98Glu + Ser130Asp  
 5 Gly 61Asp + Tyr102Pro + Ser206Glu + Thr207Asn + Ser210Glu  
 Thr 64Asn + Ser 97Asp + Pro129Asn + Val197Asp + Leu211Glu  
 Ser 99Glu + Ala181Thr + Val197Glu + Ala209Asn + Leu211Asp  
 Gly 59Asp + Val 93Ser + Asn153Ser + Val197Glu + Leu211Asp  
 Ser101Glu + Asn153Ser + Tyr208Met + Leu211Glu + Gly213Asp  
 10 Gly157Asp + Gln185Asn + Thr207Glu + Ala209Asp + Asn212Gln  
 Gly100Gln + Gly125Ser + Phe183Asp + Ser210Asp + Thr214Glu  
 Asn198Gln + Ser206Glu + Thr207Pro + Ser210Asp + Asn212Asp  
 Ala181Thr + Gln200Glu + Thr207Asp + Leu211Ala + Gly213Glu  
 Asn 60Asp + Gly 98Pro + Ser126Glu + Pro129Glu + Gln200Ser  
 15 Asp 58Glu + Ile159Pro + Ser210Glu + Asn212Asp + Thr214Gln  
 Asp 58Glu + Gly125Ser + Ala131Ser + Ser210Asp + Asn212Glu  
 Gly 59Ser + Ser101Glu + Ala131His + Ser210Glu + Asn212Glu  
 Asn 60Gln + Val 93Ala + Ser103Glu + Ser210Glu + Asn212Glu  
 Ser158Glu + Asn198Ser + Tyr208Glu + Ser210Asp + Leu211Ser  
 20 Ala 96Ser + Ser160Asp + Asn198Glu + Asn212Ser + Thr214Glu  
 Leu 94Asn + Gly 98Asp + Gly100Glu + Pro127Glu + Val197Met  
 Asn 60Ser + Gly 98Asp + Gly100Asp + Tyr102Asn + Gln200Glu  
 Leu 94Ser + Ala 96Thr + Ile105Asp + Tyr161Glu + Leu211Ile  
 Gly 95Ser + Ser 99Asp + Ser101Asp + Leu211Met + Gly213Glu  
 25 Gly 61Glu + Gly125Ser + Ser128Glu + Ser160Asp + Ala181Pro  
 Ser182Glu + Val193Met + Asn198Glu + Gly205Gln + Ala209Asp  
 Leu 94Glu + Gly100Asn + Ser104Asp + Ile105Val + Ser206Glu  
 Thr 64Asp + Ser126Asp + Gly152Asp + Pro204Gly + Leu211Asn  
 Gly 59Asp + Val 93Glu + Leu 94Pro + Ile105Met + Leu211Ser  
 30 Pro129Glu + Ser160Asp + Gln200Ser + Gly205Gln + Ser210Glu  
 Ile159Val + Ser160Glu + Phe183Glu + Pro204Gln + Ala209Asn  
 Leu 94Asp + Val197Asp + Thr207Gly + Ser210Asp + Leu211Met  
 Leu124Glu + Phe183Pro + Val197Glu + Tyr208Leu + Ser210Glu  
 Leu 94Met + Leu124Asp + Asn153Asp + Tyr161Ala + Leu211Glu  
 35 Gly 98Asp + Gly152Glu + Ala156Asp + Tyr208Met + Asn212Ser  
 Val 93Asp + Gly 98Gln + Ser126Asp + Ser130Glu + Ala209Pro  
 Asp 58Glu + Tyr102Ala + Ser126Asp + Ser130Glu + Ile159Asn  
 Ser126Asp + Ser130Glu + Val197Gly + Leu211Glu + Asn212Ser  
 Ser104Glu + Ile105Pro + Asn198Asp + Leu211His + Gly213Asp  
 40 Ser 97Asp + Gly100Glu + Gly152Pro + Tyr161Glu + Ala181Gln  
 Ser128Glu + Ala131Glu + Ser154Asp + Asn198Gln + Ala209His  
 Ser 99Asp + Pro129Ser + Ser154Glu + Ser160Asp + Pro204Gln  
 Asp 58Glu + Ile105Cys + Leu124Ser + Ser154Glu + Ser158Glu  
 Ala131Asp + Val197Glu + Gln200Glu + Thr207Pro + Thr214Gly  
 45 Ser 99Asp + Val197Asp + Asn198Gln + Gln200Glu + Thr207Gly  
 Ser101Glu + Val197Asp + Gln200Glu + Pro204Gly + Thr207Ser  
 Ala131Asp + Val197Asp + Asn198Gln + Gln200Glu + Ala209Asn  
 Gly 95Pro + Gly 98Asp + Leu124Glu + Thr207Asn + Asn212Glu  
 Gln 57Glu + Asn 60Glu + Val 93Thr + Gly 95Asn + Ser182Asp  
 50 Asn 60Asp + Ile105Gln + Ala131Thr + Thr207Ser + Ala209Glu  
 Ser103Glu + Gln200Glu + Thr207Pro + Ala209Gln + Asn212Asp  
 Gly 61Pro + Leu 94Glu + Gln200Asp + Leu211Asn + Asn212Glu  
 Gly 59Pro + Val 93Glu + Gln200Asp + Leu211Val + Asn212Asp  
 Ser 97Glu + Gly157Ser + Ala181Pro + Gln200Glu + Asn212Glu

Val 93Thr + Gly 98Gln + Tyr102Glu + Ala131Glu + Ser210Asp  
 Gly 61Glu + Ser104Asp + Phe183Ala + Thr207Gly + Ala209Glu  
 Ala156Asp + Ser160Asp + Ala181Gly + Asn198Ser + Gln200Asp  
 Gln 57Glu + Gly 61Asp + Asn153Gln + Asn198Asp + Gly205Ser  
 5 Gly100Glu + Ile105Ser + Leu124Glu + Val197Met + Ser210Asp  
 Tyr102Glu + Ser126Glu + Val193Pro + Pro204Asn + Ser210Asp  
 Gly 59Pro + Ala131Asp + Asn153Ser + Ser154Asp + Thr214Asp  
 Ser103Glu + Ser130Glu + Gln200Ser + Gly205Glu + Thr214Gly  
 Asp 58Glu + Ala156Pro + Gly157Asp + Ser160Asp + Gln200Ser  
 10 Thr 64Glu + Leu 94Asp + Ala 96Gln + Gln185Ser + Ser210Asp  
 Ser130Asp + Gly157Glu + Phe183Glu + Asn198Gln + Leu211Met  
 Asp 58Glu + Pro129Glu + Ala156His + Pro204Ser + Gly205Glu  
 Ser126Glu + Ile159Leu + Ala209Asp + Leu211Ala + Asn212Glu  
 Gly 59Asp + Ser126Asp + Ser154Glu + Phe183Leu + Leu211Val  
 15 Gly155Asn + Gly157Asp + Gln200Glu + Thr207Glu + Leu211Asn  
 Gly 59Glu + Gly 95Pro + Ala181His + Gln200Glu + Thr207Asp  
 Gln 57Glu + Pro127Gly + Gly152Ser + Gln200Asp + Thr207Asp  
 Ala 96Glu + Asn198Ser + Gln200Glu + Thr207Glu + Thr214Ser  
 Thr 64Asp + Leu124Ser + Pro129Asp + Tyr161Asn + Thr207Asp  
 20 Ser 97Asp + Tyr161Gly + Ser210Glu + Leu211Pro + Gly213Asp  
 Pro127Ser + Pro129Asn + Gly157Asp + Ser210Asp + Gly213Glu  
 Tyr102Cys + Ser130Asp + Pro204Gln + Ser210Glu + Gly213Asp  
 Ser 99Glu + Ala156Ser + Ala209Thr + Ser210Asp + Gly213Asp  
 Gly196Pro + Gln200Asp + Pro204Asp + Thr207Gln + Leu211Asn  
 25 Asp 58Glu + Ser 97Glu + Ser104Asp + Pro129Gly + Tyr161Thr  
 Asp 58Glu + Leu 94Ser + Ser 97Asp + Gly 98Pro + Gln200Asp  
 Gly 61Glu + Ser101Asp + Thr207Asp + Leu211Ile + Thr214Ser  
 Gly 61Asp + Ser101Glu + Tyr102Val + Thr207Glu + Thr214Ser  
 Gly 61Asp + Asn153Gln + Ile159Gly + Asn198Asp + Thr207Glu  
 30 Gly 61Glu + Ile159Ser + Val197Met + Asn198Glu + Thr207Asp  
 Thr 64Gln + Gly100Glu + Gln200Asn + Thr207Glu + Ser210Asp  
 Ser126Glu + Gly155Pro + Tyr161Thr + Thr207Glu + Ser210Asp  
 Tyr102Glu + Gly125Gln + Ala181Pro + Thr207Asp + Ser210Glu  
 Gly100Glu + Gly205Ser + Thr207Glu + Ser210Glu + Asn212Gln  
 35 Gly 59Ser + Gly 95Glu + Thr207Glu + Ser210Asp + Leu211Val  
 Tyr161Asp + Asn198Ser + Thr207Glu + Ser210Glu + Gly213Pro  
 Pro129Gln + Ser130Glu + Asn198Gln + Thr207Asp + Ser210Glu  
 Gln 57Asp + Phe183Gly + Pro204Gln + Thr207Glu + Ser210Asp  
 Asn 60Gln + Gly157Glu + Pro204Gly + Thr207Asp + Ser210Asp  
 40 Pro129Asp + Gly152Pro + Thr207Asp + Ser210Glu + Leu211Asn  
 Gly 61Glu + Ser 99Asp + Gly155Asn + Pro195Gln + Gln200Asn  
 Gly 98Glu + Ser101Glu + Ala181Gly + Gln185Glu + Thr207Pro  
 Gly 59Pro + Thr 64Asp + Pro129Gln + Ala181Glu + Asn198Asp  
 Gly 98Asp + Tyr161Met + Ala181Glu + Asn198Asp + Leu211Val  
 45 Ile159Glu + Val197Thr + Tyr208Gln + Ala209Glu + Thr214Asp  
 Ser126Glu + Gly157Glu + Ser182Glu + Asn198Gln + Leu211Val  
 Gly 61Asp + Ser128Glu + Ser206Asp + Tyr208Gly + Leu211Met  
 Gly 98Pro + Ser130Glu + Ser160Glu + Thr207Gly + Ser210Asp  
 Gln 57Asp + Gly125Gln + Ser130Asp + Asn153Ser + Ser160Asp  
 50 Tyr161Met + Gln200Glu + Ser206Glu + Ala209His + Gly213Asp  
 Gly 61Asp + Ala156Glu + Gly205Asn + Thr207Gln + Ser210Glu  
 Ile105Glu + Ser130Asp + Gln200Asn + Gly205Glu + Thr207Asn  
 Gln 57Ser + Asn 60Asp + Gln200Asp + Gly205Glu + Thr207Ser  
 Ser101Glu + Ser126Glu + Val197Asp + Thr207Gly + Leu211Cys



Ser101Asp + Ser126Asp + Gly152Ser + Asn198Glu + Gln200Asn  
 Gln 57Asp + Gly125Asp + Gly205Asp + Thr207Ser + Tyr208Thr  
 Leu 94Met + Ser128Asp + Ser158Glu + Asn198Gln + Ser210Asp  
 Gly 59Asp + Gly 95Pro + Ala194Gln + Asn198Asp + Thr214Asp  
 5 Gln 57Glu + Gly 61Asn + Gly 98Asp + Gly100Pro + Val193Cys  
 Thr 64Gln + Val 93Glu + Ser 97Glu + Pro129Ser + Gln200Glu  
 Gln 57Glu + Thr 64Asn + Val 93Asp + Gln200Glu + Thr207Asn  
 Gly100Asp + Gly125Ser + Ser126Glu + Gly196Gln + Val197Glu  
 Val 93Met + Gly100Asp + Ser126Glu + Tyr161Ala + Asn198Asp  
 10 Gly100Glu + Ser126Asp + Ala181His + Val197Cys + Gln200Glu  
 Asn 60Gln + Ser 99Asp + Ser103Glu + Ser210Asp + Leu211Ala  
 Gln 57Ser + Ser 99Glu + Ser103Glu + Gln200Asp + Thr207Asn  
 Gly 63Ser + Ala 96Ser + Ser210Glu + Leu211Pro + Thr214Asp  
 Pro129Gly + Val197Gln + Ser210Glu + Leu211His + Thr214Asp  
 15 Pro127Asp + Gly155Glu + Tyr161Ser + Thr207Ser + Ser210Glu  
 Asp 58Glu + Val 93His + Gly155Pro + Gln200Asn + Thr207Glu  
 Thr 64Asn + Asn198Ser + Gln200Glu + Ser206Glu + Thr214Gln  
 Gly 59Pro + Gly 61Glu + Ser154Asp + Gln200Asp + Thr214Asn  
 Ser126Asp + Pro129Ser + Ala156Asp + Ser210Asp + Leu211Ala  
 20 Ser103Glu + Ile159Leu + Pro204Glu + Gly205Asn + Ser210Asp  
 Ala131Thr + Asn153Glu + Tyr203Asn + Pro204Glu + Ser210Glu  
 Gly157Asn + Ser160Glu + Pro204Glu + Gly205Ser + Ser210Glu  
 Gly 95Gln + Gly 98Pro + Gly152Glu + Gly157Pro + Val197Asp  
 Gln 57Glu + Leu 94Pro + Gly125Ser + Asn153Glu + Leu211Glu  
 25 Gly 59Ser + Ser104Asp + Ser130Glu + Ser210Glu + Leu211Ser  
 Ser104Glu + Ser130Asp + Gly155Asn + Leu211Gly + Gly213Asp  
 Ser104Glu + Ser130Glu + Asn198Asp + Gln200Asn + Thr207Gln  
 Ser158Glu + Thr202Ser + Thr207Pro + Leu211Thr + Thr214Asp  
 Gly 59Glu + Leu 94Val + Ser101Asp + Ala131Glu + Tyr161Met  
 30 Asp 58Glu + Ser 99Glu + Tyr102Asn + Ala209Asn + Ser210Glu  
 Ser101Asp + Phe183Ser + Gln200Asp + Pro204Glu + Thr214Gln  
 Gly 61Asp + Gly 98Asn + Ser 99Glu + Ile105Ser + Val197Glu  
 Asn 60Ser + Gly125Pro + Ala156Asp + Tyr161Asp + Ser210Asp  
 Gly 59Pro + Ser128Glu + Ser154Asp + Thr207Glu + Leu211Ile  
 35 Ser103Asp + Ala156Gly + Ser182Glu + Asn198Glu + Tyr203His  
 Val 93Ala + Gly125Pro + Ser160Glu + Thr207Asp + Leu211Glu  
 Ser 97Asp + Gly152Pro + Thr207Glu + Leu211Glu + Gly213Gln  
 Gln 57Glu + Ser103Asp + Pro129Glu + Ala181Gly + Thr207Pro  
 Ser158Asp + Tyr161Pro + Ser182Asp + Thr207Gln + Ser210Asp

40

TABLE 37

Multi-loop Sextuple Mutation Variants	
	Tyr102Cys + Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln + Thr207Gly
45	Ser160Glu + Tyr203Cys + Thr207Pro + Leu211Met + Asn212Gln + Gly213Gln
	Val 93Ser + Ser103Glu + Gly125Ser + Gln185Asn + Ala194Gln + Asn198Gln
	Gly100Ser + Ile105Met + Gly157Pro + Gln200Asn + Thr207Asn + Leu211Val
50	Thr 64Glu + Leu 94Asn + Val197Cys + Thr202Gln + Thr207Gln + Leu211Val

Gly152Pro + Gly157Gln + Ala181Asp + Asn198Gln + Gln200Asn  
 + Thr207Asn  
 Gly 95Gln + Ala 96Asn + Leu124Thr + Asn153Ser + Ser154Glu  
 + Val197His  
 5 Val 93Met + Pro127Ser + Gly155Pro + Ser182Asp + Gln200Ser  
 + Gly213Ser  
 Gly 61Pro + Gly 95Pro + Ser154Glu + Ala181His + Leu211Asn  
 + Gly213Pro  
 Tyr161Thr + Pro195Asn + Gln200Asn + Thr207Gly + Tyr208Leu  
 10 + Leu211Pro  
 Leu 94Pro + Ala 96Ser + Pro127Asp + Ile159Ala + Thr207Pro  
 + Gly213Gln  
 Gly100Asn + Ile105Gln + Gly125Gln + Pro129Gly + Gln185Glu  
 + Thr207Gln  
 15 Gly 98Glu + Pro129Gly + Gly155Pro + Thr207Gly + Ala209His  
 + Thr214Gly  
 Ile105Gln + Gly152Asp + Phe183His + Ala194Gln + Gly205Ser  
 + Asn212Gln  
 Ala194Gln + Asn198Ser + Gln200Asn + Ser206Asp + Leu211His  
 20 + Thr214Gln  
 Ser 99Glu + Pro129Asn + Ala131Pro + Gln185Asn + Thr207Pro  
 + Leu211Val  
 Leu 94Val + Gly 98Gln + Leu124Gly + Val197Glu + Gln200Asn  
 + Thr207Gly  
 25 Gln 57Ser + Gly 98Asn + Tyr102Asp + Tyr161Asn + Gln200Ser  
 + Thr207Gln  
 Asn 60Gln + Gly 61Ser + Tyr102Cys + Gly152Asp + Val199Ser  
 + Pro204Asn  
 Tyr102Thr + Gly155Glu + Ala156Asn + Tyr161Pro + Gln200Asn  
 30 + Leu211Thr  
 Tyr102Glu + Gly157Pro + Phe183His + Asn198Gln + Thr207Pro  
 + Leu211Cys  
 Leu124Cys + Pro127Asn + Tyr208Met + Ser210Asp + Leu211His  
 + Gly213Gln  
 35 Gly 98Ser + Leu124Ala + Ser126Asp + Ala181Asn + Ala209Gly  
 + Gly213Gln  
 Ala 96His + Tyr102Ser + Ile105Ser + Gly157Ser + Ile159Gln  
 + Leu211Asp  
 Thr 64Pro + Ala 96Pro + Ser 97Asp + Pro127Ser + Gly205Asn  
 40 + Leu211Ser  
 Val 93Gly + Gly 98Ser + Tyr102Asn + Val197Met + Thr207Gln  
 + Ser210Asp  
 Asn 60Gln + Ala 96Gln + Asn153Glu + Thr202Gln + Gly205Asn  
 + Leu211Met  
 45 Gly 59Asn + Leu 94Val + Pro127Ser + Ile159Ala + Gln200Asn  
 + Leu211Met  
 Ile105Gln + Gly157Gln + Val193Asn + Val197Asp + Thr207Pro  
 + Tyr208Cys  
 Asn 60Gln + Gly 63Gln + Ile105Glu + Asn198Gln + Leu211Ile  
 50 + Thr214Gly  
 Gly 61Gln + Gly125Glu + Tyr161Gly + Gly205Gln + Thr207Ser  
 + Ala209Gln  
 Gly 98Ser + Ala156Gln + Tyr161Ala + Ser182Glu + Gln200Ser  
 + Thr207Gln

Gly 61Pro + Val 93Ala + Leu124Gly + Ala131Thr + Val197Glu  
 + Asn198Gln  
 Val 93Asp + Ala 96Asn + Val197His + Asn198Ser + Val199Ser  
 + Thr207Gln  
 5 Tyr102Ile + Ile159Asp + Ala181Asn + Gln200Ser + Pro204Gln  
 + Leu211His  
 Gly100Pro + Pro129Ser + Gln200Ser + Thr207Pro + Ala209Glu  
 + Ser210Glu  
 Val197Pro + Asn198Gln + Gln200Glu + Pro204Gly + Thr207Gln  
 10 + Ser210Asp  
 Ala 96Gln + Leu124Met + Ile159Ser + Gln200Glu + Pro204Gly  
 + Ser210Asp  
 Asn 60Gln + Ile159Thr + Asn198Ser + Gln200Glu + Ser210Glu  
 + Leu211Asp  
 15 Gly 61Ser + Val197Gln + Asn198Glu + Gln200Ser + Ser210Asp  
 + Leu211His  
 Gly100Asn + Ala181Pro + Asn198Asp + Thr207Ser + Ser210Glu  
 + Gly213Gln  
 Gly 98Asn + Pro129Ser + Asn198Asp + Gln200Asp + Ser210Glu  
 20 + Leu211Pro  
 Gly 63Ser + Leu 94Gln + Ile159Ala + Gly205Glu + Thr207Glu  
 + Thr214Asn  
 Asp 58Glu + Gly 61Glu + Gly 98Asn + Asn153Ser + Tyr161Asn  
 + Leu211Cys  
 25 Leu124Ser + Ile159Thr + Asn198Glu + Gln200Glu + Pro204Gly  
 + Thr207Gln  
 Gly 61Gln + Gly 95Asn + Ala194Gly + Asn198Asp + Gln200Asp  
 + Leu211Val  
 Gln 57Ser + Tyr102Gln + Asn198Glu + Ala209Glu + Ser210Glu  
 30 + Leu211Ser  
 Gly 59Gln + Gly125Pro + Ser154Glu + Gly155Asp + Tyr161Ala  
 + Ser182Asp  
 Gln 57Glu + Gly 59Asp + Thr 64Gln + Tyr208Pro + Ala209Gln  
 + Leu211Asn  
 35 Asn 60Asp + Gly 61Gln + Gly 95Glu + Gly 98Glu + Asn153Ser  
 + Ile159Leu  
 Ile105Thr + Gly152Ser + Ser158Asp + Ser160Asp + Thr207Gly  
 + Leu211Ala  
 Gly 61Gln + Thr 64Asn + Asn198Glu + Ser210Asp + Leu211Asp  
 40 + Gly213Asp  
 Leu 94Pro + Ala 96Gly + Thr207Ser + Ser210Asp + Leu211Cys  
 + Asn212Glu  
 Gly 98Glu + Gly100Asp + Ala181Pro + Tyr203Val + Pro204Asn  
 + Thr207Ser  
 45 Asp 58Glu + Gly157Ser + Gln185Asn + Val197Gln + Pro204Glu  
 + Asn212Gln  
 Gly 61Asn + Leu124Thr + Ser128Glu + Ser160Glu + Ala181Asn  
 + Thr207Gln  
 Ile105Leu + Leu124Ser + Gly125Asp + Gly152Glu + Asn153Glu  
 50 + Gly213Glu  
 Leu124Gln + Asn198Glu + Gln200Asp + Thr207Gly + Tyr208Glu  
 + Leu211Asp  
 Leu 94Ile + Gly152Ser + Gly155Glu + Ser158Asp + Ala181Gly  
 + Val197His

Asp 58Glu + Tyr161Gly + Val197Ala + Asn198Gln + Pro204Glu  
+ Ser206Asp  
Gln 57Ser + Ile159Ser + Asn198Asp + Gln200Asn + Tyr208Asn  
+ Gly213Glu  
5 Gly152Ser + Phe183Ala + Asn198Glu + Val199Ser + Leu211Cys  
+ Gly213Glu  
Leu 94Ile + Ser154Asp + Ile159Glu + Asn198Ser + Gln200Asn  
+ Leu211Met  
Ala 96Pro + Ser154Asp + Ala156His + Gly157Asn + Ser158Glu  
10 + Pro204Ser  
Gln 57Asp + Gly 59Pro + Asn 60Asp + Gly 95Pro + Thr207Ser  
+ Ala209Gln  
Thr 64Ser + Gly152Gln + Ala181His + Gln200Glu + Leu211His  
+ Asn212Glu  
15 Gln 57Asn + Gly 98Gln + Gln200Asp + Leu211Cys + Asn212Asp  
+ Gly213Ser  
Ala 96Asn + Gly100Asp + Leu124Asp + Val197Gly + Thr207Pro  
+ Gly213Ser  
Ala 96Ser + Ser154Asp + Phe183Gln + Thr207Ser + Leu211Thr  
20 + Gly213Asp  
Gly 95Asp + Gly 98Pro + Ser 99Asp + Gly100Ser + Tyr102Glu  
+ Ser103Glu  
Gln 57Asp + Asp 58Glu + Thr 64Asp + Ser 97Glu + Gln200Ser  
+ Tyr208Thr  
25 Gly125Asp + Ser130Asp + Asn153Glu + Ala156Thr + Ser160Glu  
+ Asn198Ser  
Gly 59Asn + Leu124Thr + Ile159Met + Ala209Asp + Leu211Cys  
+ Asn212Asp  
Gly 95Gln + Gly100Asn + Ile105Met + Ala181Gln + Gln200Glu  
30 + Thr207Glu  
Ile105Met + Ser126Asp + Asn153Glu + Gly205Gln + Thr207Pro  
+ Leu211Met  
Pro129Gly + Ala131His + Val197Thr + Thr207Asn + Ser210Glu  
+ Gly213Glu  
35 Gly 98Asp + Gly152Glu + Asn153Glu + Ser154Asp + Val197Ser  
+ Gln200Asn  
Asp 58Glu + Gly 61Pro + Val 93Gly + Ser 97Glu + Pro129Asn  
+ Tyr161Ile  
Ser130Asp + Gly152Ser + Val197Gly + Asn198Glu + Ser210Glu  
40 + Leu211Asp  
Tyr102Val + Gly155Asn + Ser182Asp + Val197Asp + Gln200Asp  
+ Ser210Asp  
Thr 64Gly + Ser128Glu + Gln200Glu + Thr207Ser + Ser210Asp  
+ Leu211Asp  
45 Gln 57Glu + Tyr102Ser + Thr207Pro + Ser210Asp + Leu211Asp  
+ Asn212Asp  
Pro127Asn + Gly152Glu + Ile159Asn + Asn198Asp + Gln200Asn  
+ Gly213Glu  
Asn153Ser + Gly155Ser + Ile159Thr + Asn198Asp + Ser210Glu  
50 + Thr214Asp  
Val 93Thr + Ser154Asp + Ser158Glu + Ser182Glu + Asn198Ser  
+ Thr207Ser  
Ser101Asp + Gly155Pro + Asn198Glu + Gln200Glu + Ala209Asn  
+ Ser210Asp

Ser 99Glu + Ser126Asp + Pro127Asp + Ile159Ser + Gln200Ser  
 + Leu211Met  
 Pro129Asn + Asn153Asp + Ser158Asp + Gln200Ser + Pro204Ser  
 + Gly205Ser  
 5 Val197Cys + Gly205Asp + Thr207Asp + Ser210Asp + Leu211Ile  
 + Asn212Ser  
 Gly 61Asn + Asn198Asp + Gln200Glu + Thr207Glu + Tyr208Cys  
 + Leu211His  
 Asn 60Asp + Pro127Gln + Asn153Glu + Ser154Glu + Ala156Glu  
 10 + Thr207Pro  
 Ser 99Glu + Asn153Asp + Ser154Asp + Ala156Glu + Gln185Asn  
 + Pro195Ser  
 Thr 64Asn + Ala156Asn + Val197Asp + Gln200Glu + Leu211Pro  
 + Thr214Glu  
 15 Ser126Asp + Pro127Gln + Ser128Asp + Ser160Asp + Gln200Glu  
 + Thr214Ser  
 Asn153Asp + Gln185Glu + Asn198Ser + Gln200Glu + Leu211Cys  
 + Gly213Asp  
 Ile105Gln + Leu124Glu + Gly125Glu + Ser160Asp + Ala194Gln  
 20 + Pro204Glu  
 Asp 58Glu + Leu 94Pro + Ser154Glu + Gly155Asp + Ser182Asp  
 + Asn198Gln  
 Ser 99Glu + Val197Glu + Thr207Gly + Ala209Asn + Ser210Glu  
 + Leu211Asp  
 25 Gln 57Asn + Gly125Glu + Asn153Glu + Gln200Asn + Leu211Ser  
 + Asn212Asp  
 Leu 94Ser + Gly125Gln + Ser126Glu + Val197Asp + Ser210Asp  
 + Asn212Glu  
 Thr 64Glu + Ile159His + Asn198Glu + Gln200Asp + Leu211Cys  
 30 + Thr214Gly  
 Gly 98Asp + Gly152Glu + Gly155Glu + Ala156Asp + Tyr208Met  
 + Asn212Ser  
 Gly 98Pro + Ser104Glu + Asn198Glu + Gln200Asp + Gly205Gln  
 + Asn212Asp  
 35 Asp 58Glu + Thr 64Glu + Ala 96Ser + Tyr102His + Gln200Glu  
 + Leu211Gly  
 Leu 94Ala + Gly100Gln + Phe183Asp + Val193Gln + Val197Asp  
 + Gln200Glu  
 Asn 60Gln + Thr 64Gly + Gly125Glu + Ser154Glu + Gly157Asp  
 40 + Thr207Gly  
 Val 93Met + Gly100Asp + Ser126Glu + Pro129Asp + Ala181Asn  
 + Thr214Ser  
 Gly125Asn + Ser182Glu + Phe183Tyr + Gln185Asp + Asn198Asp  
 + Asn212Glu  
 45 Leu 94Gln + Gly125Asp + Ser128Glu + Pro129Glu + Asn198Gln  
 + Ser210Glu  
 Gly 59Gln + Thr 64Asp + Asn198Gln + Gln200Asn + Ser210Asp  
 + Asn212Asp  
 Gly 59Gln + Gly125Glu + Asn198Asp + Gln200Asp + Leu211Ile  
 50 + Gly213Asp  
 Thr 64Asn + Gly125Glu + Asn153Asp + Gln185Glu + Thr207Ser  
 + Leu211Asp  
 Asp 58Glu + Gly 95Glu + Gly 98Glu + Gln185Asn + Pro204Ser  
 + Ser210Asp

Gln 57Glu + Gly 59Glu + Thr 64Asp + Gly152Ser + Ala156Glu  
+ Leu211Ser  
Asn 60Ser + Ala156Gln + Asn198Glu + Gly205Gln + Thr207Asp  
+ Leu211Asp  
5 Leu 94Ala + Ser101Asp + Ser154Glu + Ser158Asp + Ser160Asp  
+ Leu211Thr  
Asp 58Glu + Gln200Glu + Thr207Glu + Ser210Glu + Leu211Thr  
+ Gly213Pro  
Gly 61Gln + Gly152Asp + Gln200Glu + Ser210Glu + Leu211Gly  
10 + Thr214Asp  
Asn 60Glu + Tyr102Ile + Gln200Glu + Thr207Asp + Ser210Asp  
+ Asn212Ser  
Gly 59Ser + Val 93Asp + Ile159Asp + Ser160Glu + Thr207Asn  
+ Gly213Ser  
15 Thr 64Asn + Ser104Asp + Ile105Cys + Ala156Gly + Ser210Asp  
+ Leu211Asp  
Gln 57Asn + Gly 98Asp + Gly100Gln + Ser206Glu + Thr207Glu  
+ Tyr208Met  
Gln 57Asn + Ser158Asp + Ile159Glu + Phe183Met + Asn198Asp  
20 + Thr207Asn  
Gly 95Asp + Ala 96Gly + Ala131Gln + Thr207Glu + Tyr208Glu  
+ Leu211Thr  
Ser103Asp + Ser104Asp + Gln200Asn + Leu211Asn + Asn212Asp  
+ Gly213Asp  
25 Gly 61Gln + Gly 98Glu + Gly155Asp + Phe183Asp + Thr207Gln  
+ Leu211Pro  
Leu 94Ser + Gly 95Ser + Ser 97Glu + Gly 98Glu + Ala131Glu  
+ Asn212Ser  
Thr 64Asn + Tyr102Ile + Ser103Asp + Ser104Asp + Ala181Asn  
30 + Tyr208Asp  
Thr 64Gly + Tyr102Asp + Gln200Glu + Thr207Asn + Tyr208Gln  
+ Ser210Glu  
Ser 99Glu + Tyr102His + Ile159Ala + Gln200Asp + Thr207Gln  
+ Ser210Glu  
35 Thr 64Glu + Leu124Cys + Val197Glu + Gln200Asn + Ser210Asp  
+ Leu211Gly  
Thr 64Gly + Ser154Asp + Ala181Asn + Gln200Glu + Thr207Glu  
+ Ser210Asp  
Tyr102Ala + Ala181Asn + Ser182Glu + Gln200Asp + Thr207Asp  
40 + Ser210Asp  
Gly125Pro + Gly152Asn + Ser158Glu + Ile159Glu + Asn198Asp  
+ Ser210Glu  
Gln 57Ser + Asn 60Ser + Val 93Gln + Leu 94Gly + Gln200Glu  
+ Gly213Asp  
45 Gly 61Glu + Thr 64Ser + Val 93Asp + Asn198Ser + Pro204Glu  
+ Tyr208Gly  
Ser101Asp + Ser103Glu + Leu124Met + Gly155Ser + Ser210Asp  
+ Leu211Gln  
Ser 99Glu + Pro127Asp + Pro129Ser + Ser154Glu + Ser158Glu  
50 + Thr207Ser  
Gly 61Glu + Ser101Glu + Ser104Glu + Ile159Leu + Gly196Asn  
+ Asn198Gln  
Gly 59Asp + Asn 60Glu + Ser 99Glu + Val197Thr + Thr207Asp  
+ Gly213Gln

Gly 61Asn + Ala 96Glu + Gly 98Glu + Leu124Gln + Pro127Ser  
 + Gly152Glu  
 Gly125Ser + Phe183Asp + Gln185Glu + Ala209Glu + Ser210Asp  
 + Leu211His  
 5 Ser130Asp + Ile159Gly + Asn198Glu + Thr207Gln + Ser210Asp  
 + Leu211Met  
 Pro127Gly + Ser130Asp + Tyr161Thr + Asn198Glu + Ser210Glu  
 + Thr214Gln  
 Gly 98Pro + Tyr102Glu + Phe183Met + Asn198Asp + Gln200Asn  
 10 + Ser210Asp  
 Ser 99Glu + Pro127Ser + Gly155Pro + Val197Cys + Asn198Glu  
 + Leu211Glu  
 Leu 94Ser + Ser101Glu + Ile105Glu + Pro195Gln + Val197Glu  
 + Asn198Glu  
 15 Pro127Glu + Asn198Ser + Pro204Glu + Gly205Ser + Ser206Asp  
 + Thr207Pro  
 Gln 57Ser + Asn 60Asp + Gln185Asn + Asn198Glu + Leu211Thr  
 + Asn212Asp  
 Asp 58Glu + Asn 60Asp + Tyr102Ala + Ile159Ser + Ser160Glu  
 20 + Gly205Pro  
 Ala 96Gly + Ala181Asp + Asn198Asp + Thr207Glu + Ala209His  
 + Ser210Glu  
 Gln 57Asn + Asn 60Glu + Gly 95Glu + Gly100Glu + Gly125Ser  
 + Ser210Glu  
 25 Asp 58Glu + Phe183Ser + Pro204Glu + Thr207Ser + Ser210Glu  
 + Asn212Glu  
 Leu 94Asp + Pro129Glu + Ala156Ser + Tyr161Asp + Gln185Ser  
 + Asn212Ser  
 Gly 95Glu + Ser 99Asp + Ala156His + Val197Glu + Asn198Gln  
 30 + Thr207Asn  
 Thr 64Glu + Pro127Asn + Ala156Glu + Ala209Asp + Ser210Asp  
 + Leu211Asn  
 Gly 59Ser + Gly 63Pro + Pro129Ser + Gly157Glu + Gln185Asp  
 + Ser210Glu  
 35 Gln 57Ser + Gly 59Asp + Leu 94Glu + Gly125Asp + Ser160Glu  
 + Gln185Asn  
 Ile105Gln + Ser126Glu + Ser128Asp + Leu211Glu + Gly213Asp  
 + Thr214Gly  
 Pro127Ser + Ser182Glu + Gln185Glu + Asn198Asp + Thr207Pro  
 40 + Ser210Glu  
 Ala 96Asn + Ala131Pro + Ala181Asp + Thr207Asp + Ser210Asp  
 + Leu211Asp  
 Asp 58Glu + Gly 59Pro + Ser126Asp + Gly152Asp + Ser158Glu  
 + Leu211Ile  
 45 Gly155Asp + Ser182Asp + Val197Gly + Gln200Asp + Thr202Asn  
 + Leu211Glu  
 Ser126Asp + Ile159Ser + Asn198Ser + Gln200Ser + Pro204Glu  
 + Thr207Asp  
 Asn 60Ser + Gly 63Asn + Gln185Glu + Pro195Ser + Asn198Asp  
 50 + Gln200Glu  
 Gly 59Ser + Ile105Asp + Ala181Asn + Asn198Asp + Gln200Asp  
 + Leu211Thr  
 Leu 94Glu + Ser 99Asp + Gly100Gln + Ser103Asp + Gln200Glu  
 + Tyr208Met

Asp 58Glu + Leu 94Asp + Ser 99Glu + Gly100Pro + Gly157Asp  
 + Tyr203Val  
 Asp 58Glu + Thr 64Gln + Gly 98Glu + Ser 99Asp + Pro129Glu  
 + Gly155Ser  
 5 Val 93Gln + Ser101Asp + Ile105Asp + Asn198Asp + Gly205Ser  
 + Leu211Asn  
 Ser 99Glu + Ile105Ala + Ser210Glu + Leu211His + Asn212Glu  
 + Thr214Asp  
 Val 93Ala + Leu124His + Ala131Gly + Gly157Asp + Val197Asp  
 10 + Gly213Asp  
 Ile105His + Gly152Ser + Ala156Asn + Ser158Glu + Val197Asp  
 + Gly213Asp  
 Gly 61Asp + Leu 94Thr + Val197Met + Asn198Gln + Gln200Glu  
 + Gly205Glu  
 15 Tyr102Pro + Ser128Asp + Ser130Glu + Ala131Thr + Gln200Asp  
 + Leu211Asp  
 Gly 98Ser + Ser126Asp + Ser128Glu + Gly152Gln + Ser158Asp  
 + Gly205Glu  
 Ser130Asp + Phe183Ile + Thr207Gly + Tyr208Asp + Ser210Glu  
 20 + Asn212Asp  
 Asp 58Glu + Leu 94Thr + Gly100Gln + Tyr102His + Ser206Asp  
 + Thr214Pro  
 Asp 58Glu + Thr 64Glu + Ser126Glu + Pro129Ser + Ala181Gly  
 + Asn198Gln  
 25 Asn 60Ser + Leu 94Asp + Ala 96Glu + Ala181Glu + Gln185Asn  
 + Thr207Gly  
 Pro127Asp + Pro129Glu + Ala181Gln + Pro195Ser + Ser206Asp  
 + Thr214Gln  
 Gly152Gln + Ala156Asp + Thr207Asp + Ala209Glu + Leu211Pro  
 30 + Gly213Pro  
 Ser103Glu + Gly157Ser + Ser158Glu + Ser160Glu + Tyr161His  
 + Pro204Ser  
 Leu 94Asp + Gly 95Gln + Gly 98Asp + Ser126Glu + Ser154Asp  
 + Thr207Ser  
 35 Ser 97Glu + Ile105Glu + Leu124Asp + Phe183Gly + Thr207Gly  
 + Leu211Gln  
 Gly100Glu + Pro129Ser + Ala131Ser + Tyr161Val + Ser210Asp  
 + Asn212Glu  
 Gly 59Ser + Ser101Glu + Ile105Gln + Ala131His + Ser210Glu  
 40 + Asn212Glu  
 Gln 57Asn + Pro127Gln + Ala156Asp + Ile159Asp + Pro195Gly  
 + Ser206Asp  
 Val 93His + Gly100Glu + Ser126Asp + Ser128Glu + Gln185Asp  
 + Pro204Gly  
 45 Gly 95Ser + Ser 99Asp + Ser101Asp + Val197Asn + Leu211Met  
 + Gly213Glu  
 Gln 57Ser + Gly 61Glu + Ala 96Glu + Asn153Asp + Gly213Pro  
 + Thr214Glu  
 Gln 57Ser + Ala156Asp + Ser160Asp + Val197Gly + Leu211Asp  
 50 + Gly213Asp  
 Gly 98Pro + Ser104Asp + Ala181Glu + Ser182Glu + Asn198Glu  
 + Asn212Gln  
 Ile105Glu + Leu124Thr + Val197Ser + Pro204Glu + Thr207Asp  
 + Ser210Asp



Asn 60Ser + Gly 61Pro + Ser160Asp + Gln185Asp + Gln200Asn  
 + Ser210Glu  
 Gly 61Ser + Leu 94Asp + Ser104Asp + Leu124Asn + Asn198Ser  
 + Gly213Asp  
 5 Asn 60Glu + Val 93Met + Gly 95Asp + Gly 98Asn + Asn198Glu  
 + Leu211Cys  
 Gly 95Ser + Gly 98Asp + Ser 99Glu + Ser126Glu + Ser182Glu  
 + Thr207Gly  
 Asp 58Glu + Gly 98Asp + Leu124Cys + Gln200Asp + Ser210Asp  
 10 + Asn212Ser  
 Leu 94Glu + Ser 97Asp + Ser158Glu + Val197Thr + Tyr208Ser  
 + Leu211Ser  
 Ser101Glu + Ile105Glu + Ser154Glu + Gly157Glu + Asn198Ser  
 + Leu211Ala  
 15 Gly 63Ser + Gly 98Glu + Gly100Pro + Gly152Asp + Gln200Asn  
 + Gly213Asp  
 Leu 94Glu + Ser101Asp + Phe183Ala + Gln200Asn + Ser206Glu  
 + Leu211Met  
 Gln 57Glu + Asp 58Glu + Leu124Pro + Asn198Ser + Ser206Asp  
 20 + Gly213Asp  
 Gly125Glu + Ala131His + Gly205Asp + Ala209Glu + Ser210Glu  
 + Thr214Ser  
 Gly 59Ser + Gly100Glu + Ser103Asp + Asn198Ser + Tyr208Leu  
 + Ser210Glu  
 25 Asp 58Glu + Asn 60Asp + Gly155Asp + Val197Asn + Thr207Ser  
 + Thr214Asp  
 Ser 99Asp + Ser128Glu + Pro129Asp + Gly152Glu + Gln200Asn  
 + Thr207Asn  
 Pro127Gln + Asn153Glu + Ser182Asp + Val197Cys + Ser210Glu  
 30 + Leu211Pro  
 Ser104Glu + Leu124Pro + Asn153Glu + Ala156Thr + Ile159Leu  
 + Ser182Glu  
 Thr 64Asn + Tyr102Cys + Ser128Asp + Ser158Asp + Phe183Glu  
 + Asn198Ser  
 35 Ala 96Gln + Tyr161Asn + Gln185Asn + Val197Glu + Gly205Glu  
 + Ser210Asp  
 Gly 98Ser + Ser101Glu + Pro127Gly + Gln200Asp + Leu211Asp  
 + Thr214Glu  
 Gly 59Gln + Thr 64Glu + Gly 95Ser + Gly155Glu + Ser158Asp  
 40 + Val197Cys  
 Tyr102Val + Ser103Glu + Asn198Asp + Gln200Glu + Thr207Glu  
 + Ala209Asn  
 Asn 60Glu + Gly 98Gln + Asn153Glu + Ser210Asp + Leu211Ala  
 + Gly213Glu  
 45 Asp 58Glu + Gly 61Glu + Ser101Glu + Leu124Asn + Pro129Gly  
 + Ser130Asp  
 Ser126Glu + Ser130Asp + Ala131Thr + Gln200Asn + Thr202Gln  
 + Pro204Asp  
 Gly 63Gln + Ser 97Glu + Ser126Asp + Ser130Asp + Asn198Ser  
 50 + Gln200Ser  
 Val 93Gln + Leu 94Cys + Ser 97Glu + Ser101Glu + Asn212Gln  
 + Gly213Ser  
 Asp 58Glu + Gly 95Glu + Ser154Asp + Ser158Asp + Thr207Pro  
 + Tyr208Val

Asn 60Gln + Thr 64Ser + Pro129Asn + Pro204Asp + Ser210Asp  
 + Leu211Ile  
 Ser 99Asp + Ile159Gln + Val197Gly + Asn198Glu + Leu211Val  
 + Gly213Asp  
 5 Ser 97Glu + Gly100Glu + Gly152Glu + Gly155Gln + Ala181Ser  
 + Ala209His  
 Asp 58Glu + Ala 96Glu + Ser126Glu + Asn153Asp + Phe183Leu  
 + Gly205Asn  
 Gly 98Asp + Pro127Glu + Gly152Pro + Ser158Glu + Gly205Pro  
 10 + Leu211Gln  
 Ser154Glu + Gly157Asp + Ile159Asn + Asn198Glu + Gln200Asn  
 + Tyr203Asn  
 Gly 63Ser + Phe183Glu + Thr207Glu + Ser210Glu + Leu211Pro  
 + Thr214Asp  
 15 Gly100Glu + Asn153Ser + Ser154Asp + Ser160Glu + Asn198Ser  
 + Gly213Glu  
 Gln 57Glu + Ala 96His + Gly 98Pro + Ser160Asp + Pro204Asp  
 + Tyr208Gly  
 Gly 59Asp + Gly 95Pro + Ala194Gln + Asn198Asp + Gln200Asp  
 20 + Thr214Asp  
 Gly 95Glu + Val197Glu + Asn198Gln + Gln200Glu + Thr207Gln  
 + Leu211Ala  
 Gly 98Asp + Ser128Asp + Gly155Pro + Ser158Asp + Gln185Glu  
 + Gln200Ser  
 25 Gly 98Glu + Leu124Asp + Gly125Asn + Ile159Gly + Val197Asp  
 + Leu211Ala  
 Thr 64Asp + Gly152Asn + Ser160Glu + Gln185Asn + Thr207Asp  
 + Ser210Asp  
 Thr 64Asp + Gly155Asp + Ala156Ser + Val197Ser + Asn198Glu  
 30 + Leu211Asp  
 Gln 57Asn + Thr 64Pro + Ile105Glu + Ser126Glu + Ser160Asp  
 + Ser206Glu  
 Gly 98Asn + Gly157Asp + Phe183Glu + Val197Asn + Ser206Asp  
 + Thr214Glu  
 35 Ser 97Asp + Ser126Glu + Ser128Asp + Pro129Asn + Ala156Asp  
 + Leu211Gln  
 Gln 57Glu + Leu124Pro + Phe183Asn + Val197Glu + Gly205Glu  
 + Gly213Glu  
 Ser126Asp + Ser158Glu + Gln200Asp + Thr207Pro + Leu211Glu  
 40 + Asn212Ser  
 Gly 98Ser + Gly100Pro + Pro129Glu + Gly157Glu + Ser158Glu  
 + Asn198Asp  
 Gly 61Asp + Gln185Asp + Asn198Gln + Gln200Asp + Thr207Asp  
 + Leu211Ser  
 45 Asp 58Glu + Asn198Gln + Gln200Glu + Thr207Glu + Leu211Ala  
 + Asn212Glu  
 Gly 59Asp + Ser 97Glu + Pro129Asn + Gly152Pro + Ser182Glu  
 + Thr214Asp  
 Gly 59Glu + Thr 64Glu + Pro127Gln + Pro129Asn + Ala131Gly  
 50 + Asn153Glu  
 Pro127Glu + Ser130Asp + Ser154Glu + Ile159Cys + Ser210Glu  
 + Leu211Ser  
 Asp 58Glu + Val 93Asn + Ser 97Asp + Gly100Glu + Gly152Pro  
 + Asn198Glu

Gly125Glu + Gly155Asp + Gly157Asn + Gln200Glu + Gly205Asn  
 + Ser210Asp  
 Ala 96Ser + Ser103Glu + Leu124Asp + Gln200Glu + Ser210Glu  
 + Leu211Cys  
 5 Ser101Asp + Pro129Asp + Gly155Asn + Ala181Glu + Ser182Asp  
 + Gly205Pro  
 Asp 58Glu + Gly 61Glu + Ala 96His + Asn153Asp + Val197Gln  
 + Gln200Glu  
 Asn 60Asp + Ser 99Glu + Gly155Pro + Ala181Glu + Val197Asn  
 10 + Asn212Asp  
 Asn 60Gln + Gly 98Asn + Ser130Asp + Ala181Asp + Ala209Asp  
 + Asn212Asp  
 Gly 61Asp + Ala131Asp + Val197Ala + Asn198Asp + Gln200Asp  
 + Thr214Asn  
 15 Thr 64Asp + Ser 99Asp + Ser101Glu + Ala181Gln + Gln200Ser  
 + Ser210Glu  
 Leu124Ser + Tyr161Glu + Ser182Asp + Val197Glu + Thr207Gln  
 + Leu211Asn  
 Gly 95Glu + Gly 98Asp + Ser103Asp + Ala181Gln + Thr207Gln  
 20 + Asn212Asp  
 Asn 60Glu + Ser 97Glu + Ser101Glu + Leu124Ala + Pro127Glu  
 + Val197Gln  
 Val 93Asp + Ala 96Glu + Ala131Asp + Gly152Gln + Gly196Ser  
 + Val197His  
 25 Val 93Gly + Ala131Asp + Ile159Gly + Ser182Glu + Asn198Gln  
 + Gly213Asp  
 Ser130Asp + Ser182Asp + Gln185Ser + Val197Asn + Asn198Glu  
 + Ser210Asp  
 Ile159Thr + Ser182Asp + Gln200Asp + Gly205Asp + Thr207Asn  
 30 + Asn212Glu  
 Val 93Thr + Ser104Glu + Pro127Ser + Ser128Asp + Ser130Glu  
 + Asn153Glu  
 Asn 60Glu + Leu 94Asp + Gln200Ser + Gly205Asp + Ser210Asp  
 + Leu211Gln  
 35 Gly 95Asp + Ser101Asp + Ser130Glu + Asn198Asp + Gln200Ser  
 + Leu211Ala  
 Ser104Asp + Ser154Glu + Thr207Asn + Ala209Asp + Ser210Glu  
 + Leu211Asn  
 Asp 58Glu + Gly 59Glu + Ile105Leu + Ser126Asp + Phe183Asn  
 40 + Ser210Asp  
 Gly 59Glu + Asn 60Asp + Gly 95Asn + Pro129Glu + Gly152Ser  
 + Gln185Glu  
 Gly 59Asp + Ser160Glu + Gly196Ser + Gly205Ser + Ser210Asp  
 + Leu211Asp  
 45 Asn 60Glu + Ser 99Asp + Gly157Glu + Phe183Leu + Val197Asn  
 + Thr207Gln  
 Ser 99Glu + Asn198Asp + Ser206Glu + Thr207Asp + Tyr208Asn  
 + Leu211His  
 Gly 98Glu + Ser 99Asp + Ala131Pro + Asn153Glu + Tyr161Pro  
 50 + Tyr208Glu  
 Ser 97Glu + Gly 98Glu + Ser182Asp + Thr207Gln + Ser210Asp  
 + Gly213Ser  
 Ser 97Asp + Gly 98Asp + Ile105Val + Pro129Gly + Gln200Asp  
 + Gly205Glu

Asp 58Glu + Ser103Asp + Ser104Asp + Phe183Gly + Val197Pro  
 + Asn198Asp  
 Asn 60Ser + Ser 99Glu + Gly100Asp + Pro129Gly + Gly205Asp  
 + Ser210Glu  
 5 Ser 99Glu + Ser154Asp + Tyr161Cys + Val197Ser + Gln200Asp  
 + Ser210Asp  
 Ser101Glu + Gly155Asp + Gln185Ser + Asn198Gln + Gln200Asp  
 + Ser210Glu  
 Gly 59Asp + Tyr102Asn + Ser158Asp + Gln200Asp + Tyr208Val  
 10 + Ser210Glu  
 Gly 59Asp + Ala156Gln + Ile159Asp + Phe183Gly + Gln200Asp  
 + Ser210Asp  
 Pro127Ser + Ser160Asp + Ser182Asp + Val197Gln + Gln200Glu  
 + Ser210Asp  
 15 Gln 57Asn + Val 93Asp + Pro127Glu + Asn198Gln + Gln200Glu  
 + Ser210Glu  
 Leu 94Glu + Pro129Gln + Ser154Asp + Val197His + Gln200Asp  
 + Ser210Glu  
 Ala 96Ser + Gly 98Gln + Ser126Asp + Ser154Asp + Val197Thr  
 20 + Thr207Asp  
 Gln 57Ser + Ser104Asp + Gly125Asp + Ser160Glu + Gly205Pro  
 + Ser210Glu  
 Leu 94Gln + Ser 99Asp + Gly155Pro + Val193Asn + Gln200Asp  
 + Thr207Asp  
 25 Gly 95Glu + Gln200Glu + Thr202Ser + Thr207Asp + Leu211Gln  
 + Thr214Gln  
 Thr 64Gln + Ala 96Thr + Ser104Asp + Gln200Asp + Thr207Asp  
 + Thr214Pro  
 Thr 64Glu + Tyr102Cys + Ala156Ser + Phe183Glu + Pro195Gln  
 30 + Thr207Glu  
 Gly 98Glu + Val197His + Thr207Gln + Ala209Ser + Ser210Asp  
 + Gly213Asp  
 Gly 95Ser + Gly157Asp + Phe183Glu + Thr207Asp + Ala209Gln  
 + Ser210Asp  
 35 Asp 58Glu + Val 93Asp + Ser128Glu + Ala131Glu + Gly196Gln  
 + Pro204Asn  
 Gln 57Ser + Pro127Glu + Gln185Asn + Asn198Asp + Gln200Glu  
 + Ser206Glu  
 Val 93Glu + Gly152Asn + Thr207Glu + Ser210Asp + Leu211Ser  
 40 + Gly213Glu  
 Thr 64Gly + Ser 99Glu + Ser103Glu + Pro127Asn + Gly152Glu  
 + Ser158Glu  
 Gly157Asn + Ser158Glu + Asn198Asp + Ser206Asp + Thr207Gln  
 + Ser210Asp  
 45 Ala 96Asp + Ser126Glu + Ser160Glu + Tyr161Leu + Ser210Glu  
 + Gly213Ser  
 Asp 58Glu + Ala 96Ser + Ser101Asp + Ser104Glu + Ala209Gln  
 + Ser210Asp  
 Gly 61Asp + Thr 64Gln + Val 93Cys + Ile105Ser + Leu211Val  
 50 + Asn212Glu  
 Gly 61Glu + Phe183Ser + Tyr203Thr + Thr207Asn + Tyr208Gly  
 + Asn212Glu  
 Ser 97Glu + Ile105Cys + Pro127Ser + Ser160Asp + Asn198Glu  
 + Ser210Asp

Leu 94Asp + Ser130Glu + Asn198Asp + Pro204Asn + Thr207Gln  
 + Ser210Asp  
 Ile105Asp + Leu124Pro + Ile159Leu + Asn198Glu + Ser206Asp  
 + Leu211Glu  
 5 Tyr102Gly + Ala131Ser + Ser182Glu + Phe183Pro + Thr207Glu  
 + Ser210Asp  
 Asn 60Gln + Gly 61Glu + Ser 97Asp + Gly125Glu + Tyr208Pro  
 + Ser210Asp  
 Gly 98Gln + Ser160Glu + Ala181Thr + Asn198Asp + Ser206Asp  
 10 + Asn212Glu  
 Leu124Ala + Ser128Glu + Asn153Gln + Gly205Asp + Thr207Glu  
 + Asn212Glu  
 Gly 59Glu + Gly100Pro + Ser103Glu + Ile105Glu + Val197Gly  
 + Ser210Glu  
 15 Asn 60Glu + Ser 97Glu + Pro127Asp + Val193Met + Thr207Asp  
 + Ala209Asn  
 Thr 64Gln + Ala 96Asn + Ser101Glu + Ser130Asp + Asn198Ser  
 + Leu211Asp  
 Gly 61Pro + Leu 94Glu + Ser103Asp + Ile159Pro + Leu211Asn  
 20 + Asn212Glu  
 Val 93Cys + Leu 94Asp + Gly100Ser + Ser103Glu + Ser206Asp  
 + Tyr208Gly  
 Ser130Asp + Gly152Glu + Ser154Glu + Gly157Ser + Ala181Pro  
 + Asn198Glu  
 25 Gly 59Glu + Ser130Asp + Ser154Asp + Gln185Asp + Thr207Gly  
 + Leu211Pro  
 Gln 57Glu + Leu 94Ala + Ser101Asp + Ser126Glu + Ala131Gln  
 + Ser154Asp  
 Ser103Asp + Ile105Met + Gly125Asn + Ser160Asp + Val197Ser  
 + Thr214Asp  
 30 Asp 58Glu + Val 93Asp + Gly125Ser + Gly152Ser + Val193His  
 + Gln200Glu  
 Asp 58Glu + Gly 98Asp + Leu124Ser + Gly125Glu + Tyr161Met  
 + Gln200Glu  
 35 Gly 61Asp + Leu 94Ile + Asn153Asp + Ser158Asp + Val197Ala  
 + Ser210Glu  
 Asn 60Ser + Val 93Ser + Ser103Glu + Ser160Glu + Asn198Asp  
 + Gln200Asp  
 Asn 60Gln + Ser 97Asp + Ser128Asp + Asn198Glu + Gln200Asp  
 + Ala209Gly  
 40 Val 93Ser + Tyr102Asn + Ser104Asp + Gly152Asp + Asn198Glu  
 + Gln200Glu  
 Gly 59Asp + Ile105Met + Ser158Glu + Ile159Ser + Asn198Glu  
 + Gln200Asp  
 45 Gly 59Glu + Ser101Glu + Ile159Cys + Asn198Glu + Val199Gln  
 + Gln200Asp  
 Gly 61Asp + Pro127Asp + Gln185Asp + Asn198Ser + Gln200Ser  
 + Ser210Asp  
 Gly100Glu + Ser104Glu + Ser182Glu + Thr207Gly + Tyr208Met  
 + Ser210Glu  
 50 Thr 64Gly + Gly157Asp + Ser182Glu + Val197Gln + Asn198Ser  
 + Ser210Asp  
 Ser128Glu + Ser130Asp + Tyr161Val + Asn198Glu + Ser206Glu  
 + Gly213Asn

Gly 59Gln + Thr 64Asp + Ser 99Glu + Asn198Asp + Pro204Glu  
 + Thr207Asn  
 Gly 95Glu + Ser126Asp + Gln185Ser + Val197Cys + Gln200Glu  
 + Leu211Glu  
 5 Gly 98Pro + Ser 99Glu + Ser158Asp + Gln200Asp + Ala209Ser  
 + Leu211Asp  
 Ser101Glu + Pro129Glu + Asn153Ser + Ser160Asp + Thr207Glu  
 + Leu211Cys  
 10 Asn 60Glu + Gly 98Asp + Pro127Asp + Ala131Gln + Ala181Asp  
 + Asn198Ser  
 Asp 58Glu + Ser126Asp + Gly157Asn + Thr207Ser + Leu211Asp  
 + Gly213Asp  
 Ser104Asp + Leu124Gly + Asn153Asp + Ser182Glu + Asn198Glu  
 + Thr207Asn  
 15 Gly 95Ser + Ser103Glu + Pro129Glu + Ser160Asp + Gln200Asp  
 + Leu211Pro  
 Gly 59Glu + Gly125Asp + Pro127Glu + Ala131His + Ala209Asp  
 + Leu211Ile  
 Pro127Glu + Gly157Asn + Pro204Gly + Thr207Asp + Ala209Asp  
 20 + Thr214Asp  
 Gln 57Glu + Leu 94Glu + Ser103Asp + Gly152Asp + Gly205Ser  
 + Leu211Ala  
 Ala 96Glu + Ser103Asp + Gly152Gln + Ser158Glu + Ser160Asp  
 + Thr214Asn  
 25 Gly 61Glu + Tyr102His + Asn153Asp + Phe183His + Gln185Asn  
 + Ser210Asp  
 Ser 97Asp + Ser126Asp + Pro129Glu + Val197His + Asn198Asp  
 + Gln200Asn  
 Asn 60Glu + Ala 96Pro + Ile105Asp + Leu124Ala + Ser130Glu  
 30 + Ile159Ser  
 Gln 57Glu + Pro127Gly + Ser128Glu + Ala131Ser + Ser210Asp  
 + Asn212Glu  
 Ala 96Glu + Tyr102Asp + Ser128Glu + Ala156Pro + Tyr161Val  
 + Leu211Thr  
 35 Gln 57Ser + Asp 58Glu + Ser 99Asp + Gln200Glu + Tyr208Ser  
 + Asn212Glu  
 Gly 59Asp + Leu 94Met + Ile105Ala + Gly152Glu + Ser182Glu  
 + Gln185Ser  
 Asn 60Glu + Ala156Gly + Val197Asp + Asn198Ser + Gln200Glu  
 40 + Ser206Asp  
 Gly100Ser + Ser101Asp + Gly125Gln + Ser126Asp + Pro127Asn  
 + Asn198Glu  
 Ala 96Glu + Ser 99Asp + Ser160Asp + Ala181Gly + Gly205Ser  
 + Ser210Asp  
 45 Leu124Asp + Gly125Pro + Ser130Glu + Ser154Asp + Gly205Gln  
 + Ala209Thr  
 Ser101Glu + Gly152Glu + Gly155Glu + Asn198Gln + Gln200Asp  
 + Asn212Ser  
 Gly 95Asn + Ser103Asp + Gly152Glu + Gly155Asp + Tyr208Val  
 50 + Ser210Glu  
 Ser 99Glu + Gly100Ser + Gly125Asn + Gly152Asp + Gly155Asp  
 + Gly205Asp  
 Ala 96Glu + Ser160Asp + Tyr161Met + Phe183Leu + Gln185Asp  
 + Gly205Asp

Leu 94Glu + Ser104Asp + Gly157Glu + Asn198Gln + Thr207Asn  
 + Ala209Asp  
 Thr 64Gln + Ser101Glu + Ile105Pro + Gly125Ser + Pro129Glu  
 + Thr214Asn  
 5 Gly 98Gln + Tyr102Gln + Gly125Asn + Ser126Glu + Ser158Glu  
 + Asn198Glu  
 Leu 94Asp + Tyr161Ile + Ala181Gly + Phe183Asp + Gln200Glu  
 + Thr214Asp

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10 TABLE 38  
 Preferred Subtilisin 309 Variants

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	--Single Mutation--
15	Thr207Glu Ser210Glu Ser210Asp Ser210Gly Val197Glu
20	--Double Mutation--
	Gln200Glu + Ser210Glu Val199Leu + Ser210Glu Val199Leu + Ser210Asp Pro204Ala + Ala209Thr
25	Thr207Glu + Ser210Glu Tyr208Phe + Leu211Asn Ala194Glu + Ser210Glu Gln200Glu + Tyr211Asn Gln206Glu + Thr207Glu
30	--Triple Mutation--
	Gln200Pro + Gly205Ala + Ser210Glu Thr207Glu + Ser210Glu + leu211Asn Val199Ile + Pro204Asn + Thr207Glu
35	Gln200Glu + Ser210Glu + Leu211Asp Gln200Glu + Thr207Glu + Leu211Glu Gln200Glu + Thr207Gln + Ser210Glu
40	--Quadruple Mutation--
	Pro204Ala + Thr207Glu + Ser210Glu + Leu211Asn Gln206Glu + Thr207Glu + Ser210Glu + Leu211Gly
45	--Quintuple Mutation--
	Val197Leu + Pro204Ala + Thr207Glu + Ser210Glu + Leu217Asp Asn198Glu + Gln200Glu + Thr207Glu + Ser210Glu + Leu211Asp

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## II. Cleaning Compositions

In another embodiment of the present invention, an effective amount of one or more enzyme variants of the present invention are included in compositions useful for cleaning a variety of surfaces in need of proteinaceous

stain removal. Such cleaning compositions include detergent compositions for cleaning hard surfaces, unlimited in form (e.g., liquid and granular); detergent compositions for cleaning fabrics, unlimited in form (e.g., granular, liquid and bar formulations); dishwashing compositions (unlimited in form); oral cleaning compositions, unlimited in form (e.g., dentifrice, toothpaste and mouthwash formulations); denture cleaning compositions, unlimited in form (e.g., liquid, tablet); and contact lens cleaning compositions, unlimited in form (e.g., liquid, tablet). As used herein, "effective amount of enzyme variant" refers to the quantity of enzyme variant necessary to achieve the enzymatic activity necessary in the specific cleaning composition. Such effective amounts are readily ascertained by one of ordinary skill in the art and is based on many factors, such as the particular enzyme variant used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (e.g., granular, bar) composition is required, and the like. Preferably the cleaning compositions of the present invention comprise from about 0.0001% to about 10% of one or more enzyme variants of the present invention, more preferably from about 0.001% to about 1%, more preferably still from about 0.01% to about 0.1%. Several examples of various cleaning compositions wherein the enzyme variants of the present invention may be employed are discussed in further detail below. All parts, percentages and ratios used herein are by weight unless otherwise specified.

As used herein, "non-fabric cleaning compositions" include hard surface cleaning compositions, dishwashing compositions, oral cleaning compositions, denture cleaning compositions and contact lens cleaning compositions.

A. Cleaning Compositions for Hard Surfaces, Dishes and Fabrics

The enzymes of the present invention can be used in any detergent composition where high sudsing and good insoluble substrate removal are desired. Thus the enzyme variants of the present invention can be used with various conventional ingredients to provide fully-formulated hard-surface cleaners, dishwashing compositions, fabric laundering compositions and the like. Such compositions can be in the form of liquids, granules, bars and the like. Such compositions can be formulated as modern "concentrated" detergents which contain as much as 30%-60% by weight of surfactants.

The cleaning compositions herein can optionally, and preferably, contain various anionic, nonionic, zwitterionic, etc., surfactants. Such surfactants are typically present at levels of from about 5% to about 35% of the compositions.

Nonlimiting examples of surfactants useful herein include the



conventional C<sub>11</sub>-C<sub>18</sub> alkyl benzene sulfonates and primary and random alkyl sulfates, the C<sub>10</sub>-C<sub>18</sub> secondary (2,3) alkyl sulfates of the formulas CH<sub>3</sub>(CH<sub>2</sub>)<sub>x</sub>(CHOSO<sub>3</sub><sup>-</sup>M<sup>+</sup>)CH<sub>3</sub> and CH<sub>3</sub>(CH<sub>2</sub>)<sub>y</sub>(CHOSO<sub>3</sub><sup>-</sup>M<sup>+</sup>)CH<sub>2</sub>CH<sub>3</sub> wherein x and (y+1) are integers of at least about 7, preferably at least about 9, and M is a water-solubilizing cation, especially sodium, the C<sub>10</sub>-C<sub>18</sub> alkyl alkoxy sulfates (especially EO 1-5 ethoxy sulfates), C<sub>10</sub>-C<sub>18</sub> alkyl alkoxy carboxylates (especially the EO 1-5 ethoxycarboxylates), the C<sub>10</sub>-C<sub>18</sub> alkyl polyglycosides, and their corresponding sulfated polyglycosides, C<sub>12</sub>-C<sub>18</sub> alpha-sulfonated fatty acid esters, C<sub>12</sub>-C<sub>18</sub> alkyl and alkyl phenol alkoxyates (especially ethoxylates and mixed ethoxy/propoxy), C<sub>12</sub>-C<sub>18</sub> betaines and sulfobetaines ("sultaines"), C<sub>10</sub>-C<sub>18</sub> amine oxides, and the like. The alkyl alkoxy sulfates (AES) and alkyl alkoxy carboxylates (AEC) are preferred herein. (Use of such surfactants in combination with the aforesaid amine oxide and/or betaine or sultaine surfactants is also preferred, depending on the desires of the formulator.) Other conventional useful surfactants are listed in standard texts. Particularly useful surfactants include the C<sub>10</sub>-C<sub>18</sub> N-methyl glucamides disclosed in US Patent 5, 194,639, Connor et al., issued March 16, 1993, incorporated herein by reference.

A wide variety of other ingredients useful in detergent cleaning compositions can be included in the compositions herein, including other active ingredients, carriers, hydrotropes, processing aids, dyes or pigments, solvents for liquid formulations, etc. If an additional increment of sudsing is desired, suds boosters such as the C<sub>10</sub>-C<sub>16</sub> alkylamidates can be incorporated into the compositions, typically at about 1% to about 10% levels. The C<sub>10</sub>-C<sub>14</sub> monoethanol and diethanol amides illustrate a typical class of such suds boosters. Use of such suds boosters with high sudsing adjunct surfactants such as the amine oxides, betaines and sultaines noted above is also advantageous. If desired, soluble magnesium salts such as MgCl<sub>2</sub>, MgSO<sub>4</sub>, and the like, can be added at levels of, typically, from about 0.1% to about 2%, to provide additionally sudsing.

The liquid detergent compositions herein can contain water and other solvents as carriers. Low molecular weight primary or secondary alcohols exemplified by methanol, ethanol, propanol, and isopropanol are suitable. Monohydric alcohols are preferred for solubilizing surfactants, but polyols such as those containing from about 2 to about 6 carbon atoms and from about 2 to about 6 hydroxy groups (e.g., 1,3-propanediol, ethylene glycol, glycerine, and 1,2-propanediol) can also be used. The compositions may contain from about 5% to about 90%, typically from about 10% to about 50% of such carriers.

The detergent compositions herein will preferably be formulated such that during use in aqueous cleaning operations, the wash water will have a pH between about 6.8 and about 11.0. Finished products thus are typically formulated at this range. Techniques for controlling pH at recommended  
5 usage levels include the use of buffers, alkalis, acids, etc., and are well known to those skilled in the art.

When formulating the hard surface cleaning compositions and fabric cleaning compositions of the present invention, the formulator may wish to employ various builders at levels from about 5% to about 50% by weight.  
10 Typical builders include the 1-10 micron zeolites, polycarboxylates such as citrate and oxydisuccinates, layered silicates, phosphates, and the like. Other conventional builders are listed in standard formularies.

Likewise, the formulator may wish to employ various additional enzymes, such as cellulases, lipases, amylases and proteases in such  
15 compositions, typically at levels of from about 0.001% to about 1% by weight. Various detergent and fabric care enzymes are well-known in the laundry detergent art.

Various bleaching compounds, such as the percarbonates, perborates and the like, can be used in such compositions, typically at levels from about  
20 1% to about 15% by weight. If desired, such compositions can also contain bleach activators such as tetraacetyl ethylenediamine, nonanoyloxybenzene sulfonate, and the like, which are also known in the art. Usage levels typically range from about 1% to about 10% by weight.

Various soil release agents, especially of the anionic oligoester type,  
25 various chelating agents, especially the aminophosphonates and ethylenediaminedisuccinates, various clay soil removal agents, especially ethoxylated tetraethylene pentamine, various dispersing agents, especially polyacrylates and polyaspartates, various brighteners, especially anionic brighteners, various suds suppressors, especially silicones and secondary  
30 alcohols, various fabric softeners, especially smectite clays, and the like can all be used in such compositions at levels ranging from about 1% to about 35% by weight. Standard formularies and published patents contain multiple, detailed descriptions of such conventional materials.

Enzyme stabilizers may also be used in the cleaning compositions of  
35 the present invention. Such enzyme stabilizers include propylene glycol (preferably from about 1% to about 10%), sodium formate (preferably from about 0.1% to about 1%) and calcium formate (preferably from about 0.1% to about 1%).

1. Hard surface cleaning compositions

As used herein "hard surface cleaning composition" refers to liquid and granular detergent compositions for cleaning hard surfaces such as floors, walls, bathroom tile, and the like. Hard surface cleaning compositions of the present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about .01% to about 5%, more preferably still from about .05% to about 1% by weight of active enzyme of the composition. In addition to comprising one or more enzyme variants of the present invention, such hard surface cleaning compositions typically comprise a surfactant and a water-soluble sequestering builder. In certain specialized products such as spray window cleaners, however, the surfactants are sometimes not used since they may produce a filmy/streaky residue on the glass surface.

The surfactant component, when present, may comprise as little as 0.1% of the compositions herein, but typically the compositions will contain from about 0.25% to about 10%, more preferably from about 1% to about 5% of surfactant.

Typically the compositions will contain from about 0.5% to about 50% of a detergency builder, preferably from about 1% to about 10%. Preferably the pH should be in the range of about 8 to 12. Conventional pH adjustment agents such as sodium hydroxide, sodium carbonate or hydrochloric acid can be used if adjustment is necessary.

Solvents may be included in the compositions. Useful solvents include, but are not limited to, glycol ethers such as diethyleneglycol monohexyl ether, diethyleneglycol monobutyl ether, ethyleneglycol monobutyl ether, ethyleneglycol monohexyl ether, propyleneglycol monobutyl ether, dipropyleneglycol monobutyl ether, and diols such as 2,2,4-trimethyl-1,3-pentanediol and 2-ethyl-1,3-hexanediol. When used, such solvents are typically present at levels of from about 0.5% to about 15%, preferably from about 3% to about 11%.

Additionally, highly volatile solvents such as isopropanol or ethanol can be used in the present compositions to facilitate faster evaporation of the composition from surfaces when the surface is not rinsed after "full strength" application of the composition to the surface. When used, volatile solvents are typically present at levels of from about 2% to about 12% in the compositions.

The hard surface cleaning composition embodiment of the present invention is illustrated by the following examples.

## Examples 7-12

## Liquid Hard Surface Cleaning Compositions

		Example No.					
Component		7	8	9	10	11	12
5	Thr207Glu	0.05	0.50	0.02	0.03	0.10	0.03
	Val199Leu + Ser210Asp	—	—	-	-	0.20	0.02
	Na <sub>2</sub> DIDA*						
	EDTA**	—	—	2.90	2.90	—	—
	Na Citrate	—	—	—	—	2.90	2.90
10	NaC <sub>12</sub> Alkyl-benzene sulfonate	1.95	—	1.95	—	1.95	—
	NaC <sub>12</sub> Alkylsulfate	—	2.20	—	2.20	—	2.20
	NaC <sub>12</sub> (ethoxy)*** sulfate	—	2.20	—	2.20	—	2.20
15	C <sub>12</sub> Dimethylamine oxide	—	0.50	—	0.50	—	0.50
	Na Cumene sulfonate	1.30	—	1.30	—	1.30	—
	Hexyl Carbitol***	6.30	6.30	6.30	6.30	6.30	6.30
	Water****	balance to 100%					
20	*Disodium N-diethyleneglycol-N,N-iminodiacetate						
	**Na <sub>4</sub> ethylenediamine diacetic acid						
	***Diethyleneglycol monohexyl ether						
	****All formulas adjusted to pH 7						

25 In Examples 7-10, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Thr207Glu, with substantially similar results.

In Examples 11-12, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val199Leu + Ser210Asp, with substantially similar results.

Examples 13-18  
Spray Compositions for Cleaning Hard Surfaces  
and Removing Household Mildew

5	Component	Example No.					
		13	14	15	16	17	18
	Gln200Glu + Thr207Glu	0.50	0.05	0.60	0.30	0.20	0.30
	Ser210Glu	-	-	-	-	0.30	0.10
	Sodium octyl sulfate	2.00	2.00	2.00	2.00	2.00	2.00
	Sodium dodecyl sulfate	4.00	4.00	4.00	4.00	4.00	4.00
10	Sodium hydroxide	0.80	0.80	0.80	0.80	0.80	0.80
	Silicate (Na)	0.04	0.04	0.04	0.04	0.04	0.04
	Perfume	0.35	0.35	0.35	0.35	0.35	0.35
	Water	balance to 100%					

Product pH is about 7.

15 In Examples 13-16, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Glu + Thr207Glu, with substantially similar results.

In Examples 17-18, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Glu + Thr207Glu and Ser210Glu, with substantially similar results.

20 2. Dishwashing Compositions

In another embodiment of the present invention, dishwashing compositions comprise one or more enzyme variants of the present invention. As used herein, "dishwashing composition" refers to all forms for compositions for cleaning dishes, including but not limited to, granular and liquid forms. The dishwashing composition embodiment of the present invention is illustrated by the following examples.

Examples 19-24  
Dishwashing Composition

		Example No.					
Component		19	20	21	22	23	24
5	Gln200Pro + Gly205Ala + Ser210Asp	0.05	0.50	0.02	0.40	0.10	0.03
	Val199Leu + Ser210Asp	-	-	-	-	0.40	0.02
	C <sub>12</sub> -C <sub>14</sub> N-methyl-glucamide	0.90	0.90	0.90	0.90	0.90	0.90
10	C <sub>12</sub> ethoxy (1) sulfate	12.00	12.00	12.00	12.00	12.00	12.00
	2-methyl undecanoic acid	4.50	4.50	4.50	4.50	4.50	4.50
	C <sub>12</sub> ethoxy (2) carboxylate	4.50	4.50	4.50	4.50	4.50	4.50
	C <sub>12</sub> alcohol ethoxylate (4)	3.00	3.00	3.00	3.00	3.00	3.00
	C <sub>12</sub> amine oxide	3.00	3.00	3.00	3.00	3.00	3.00
15	Sodium cumene sulfonate	2.00	2.00	2.00	2.00	2.00	2.00
	Ethanol	4.00	4.00	4.00	4.00	4.00	4.00
	Mg <sup>++</sup> (as MgCl <sub>2</sub> )	0.20	0.20	0.20	0.20	0.20	0.20
	Ca <sup>++</sup> (as CaCl <sub>2</sub> )	0.40	0.40	0.40	0.40	0.40	0.40
	Water	balance to 100%					

20 Product pH is adjusted to 7.

In Examples 19-22, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Pro + Gly205Ala + Ser210Asp, with substantially similar results.

25 In Examples 23-24, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Pro + Gly205Ala + Ser210Asp and Val199Leu + Ser210Asp, with substantially similar results.

### 3. Fabric cleaning compositions

30 In another embodiment of the present invention, fabric cleaning compositions comprise one or more enzyme variants of the present invention. As used herein, "fabric cleaning composition" refers to all forms for detergent compositions for cleaning fabrics, including but not limited to, granular, liquid and bar forms. Preferred fabric cleaning compositions are those in the liquid form.

#### 35 a. Granular fabric cleaning compositions

The granular fabric cleaning compositions of the present invention contain an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from

about 0.005% to about 5%, more preferably from about 0.01% to about 1% by weight of active enzyme of the composition. In addition to one or more enzyme variants, the granular fabric cleaning compositions typically comprise at least one surfactant, one or more builders, and, in some cases, a bleaching agent.

The granular fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

## Examples 25-28

## Granular Fabric Cleaning Composition

Component	Example No.			
	25	26	27	28
Ser99Asp	0.10	0.20	0.03	0.05
Ser99Gly	-	-	0.02	0.05
C <sub>13</sub> linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
Sodium carbonate	23.00	23.00	23.00	23.00
Sodium silicate	14.00	14.00	14.00	14.00
Zeolite	8.20	8.20	8.20	8.20
Chelant (diethylenetriamine-pentaacetic acid)	0.40	0.40	0.40	0.40
Sodium sulfate	5.50	5.50	5.50	5.50
Water	balance to 100%			

In Examples 25-26, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Ser99Asp, with substantially similar results.

In Examples 27-28, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Ser99Asp and Ser99Gly, with substantially similar results.

Examples 29-32  
Granular Fabric Cleaning Composition

	Component	Example No.			
		29	30	31	32
5	Gln200Glu + Thr207Glu + Ser210Glu	0.10	0.20	0.03	0.05
	Asn74Asp + Pro204Ala + Thr207Glu	-	-	0.02	0.05
	C <sub>12</sub> alkyl benzene sulfonate	12.00	12.00	12.00	12.00
	Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
	2-butyl octanoic acid	4.00	4.00	4.00	4.00
10	C <sub>12</sub> -C <sub>14</sub> secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
	Sodium citrate	5.00	5.00	5.00	5.00
	Optical brightener	0.10	0.10	0.10	0.10
	Sodium sulfate	17.00	17.00	17.00	17.00
15	Water and minors	balance to 100%			

In Examples 29-30, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Glu + Thr207Glu + Ser210Glu, with substantially similar results.

In Examples 31-32, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Glu + Thr207Glu + Ser210Glu and Asn74Asp + Pro204Ala + Thr207Glu, with substantially similar results.

Examples 33-36  
Granular Fabric Cleaning Composition

	Component	Example No.			
		33	34	35	36
25	Leu 94Gly + Gln200Glu	0.10	0.20	0.03	0.05
	Gln 57Ser + Leu 94Gly + Gln200Glu	-	-	0.02	0.05
	C <sub>13</sub> linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
30	Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
	Sodium carbonate	23.00	23.00	23.00	23.00
	Sodium silicate	14.00	14.00	14.00	14.00
	Zeolite	8.20	8.20	8.20	8.20
35	Chelant (diethylenetriamine-pentaacetic acid)	0.40	0.40	0.40	0.40
	Sodium sulfate	5.50	5.50	5.50	5.50
	Water	balance to 100%			



In Examples 33-34, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Leu 94Gly + Gln200Glu, with substantially similar results.

In Examples 35-36, any combination of the subtilisin 309 variants  
5 recited in Tables 3-38, among others, are substituted for Leu 94Gly + Gln200Glu and Gln 57Ser + Leu 94Gly + Gln200Glu, with substantially similar results.

Examples 37-40  
Granular Fabric Cleaning Composition

10	Component	Example No.			
		37	38	39	40
	Asn74His + Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu	0.10	0.20	0.03	0.05
15	Val 93Gln + Tyr102Cys + Ser154Glu + Asn198Gln + Thr207Gly	-	-	0.02	0.05
	C <sub>12</sub> alkyl benzene sulfonate	12.00	12.00	12.00	12.00
	Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
	2-butyl octanoic acid	4.00	4.00	4.00	4.00
20	C <sub>12</sub> -C <sub>14</sub> secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
	Sodium citrate	5.00	5.00	5.00	5.00
	Optical brightener	0.10	0.10	0.10	0.10
	Sodium sulfate	17.00	17.00	17.00	17.00
	Water and minors	balance to 100%			

25 In Examples 37-38, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Asn74His + Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu, with substantially similar results.

In Examples 39-40, any combination of the subtilisin 309 variants  
30 recited in Tables 3-38, among others, are substituted for Asn74His + Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu and Val 93Gln + Tyr102Cys + Ser154Glu + Asn198Gln + Thr207Gly, with substantially similar results.

Examples 41-42  
Granular Fabric Cleaning Composition

		Example No.	
Component		41	42
5	Linear alkyl benzene sulphonate	11.4	10.70
	Tallow alkyl sulphate	1.80	2.40
	C <sub>14-15</sub> alkyl sulphate	3.00	3.10
	C <sub>14-15</sub> alcohol 7 times ethoxylated	4.00	4.00
	Tallow alcohol 11 times ethoxylated	1.80	1.80
10	Dispersant	0.07	0.1
	Silicone fluid	0.80	0.80
	Trisodium citrate	14.00	15.00
	Citric acid	3.00	2.50
	Zeolite	32.50	32.10
15	Maleic acid acrylic acid copolymer	5.00	5.00
	Diethylene triamine penta methylene phosphonic acid	1.00	0.20
	Ser210Asp	0.30	0.30
	Lipase	0.36	0.40
20	Amylase	0.30	0.30
	Sodium silicate	2.00	2.50
	Sodium sulphate	3.50	5.20
	Polyvinyl pyrrolidone	0.30	0.50
	Perborate	0.5	1
25	Phenol sulphonate	0.1	0.2
	Peroxidase	0.1	0.1
	Minors	Up to 100	Up to 100

Examples 43-44  
Granular Fabric Cleaning Composition

		Example No.	
Component		43	44
5	Sodium linear C <sub>12</sub> alkyl benzene-sulfonate	6.5	8.0
	Sodium sulfate	15.0	18.0
	Zeolite A	26.0	22.0
	Sodium nitrilotriacetate	5.0	5.0
	Polyvinyl pyrrolidone	0.5	0.7
10	Tetraacetylene diamine	3.0	3.0
	Boric acid	4.0	-
	Perborate	0.5	1
	Phenol sulphonate	0.1	0.2
	Val199Leu + Ser210Glu	0.4	0.4
15	Fillers (e.g., silicates; carbonates; perfumes; water)	Up to 100	Up to 100

Example 45  
Compact Granular Fabric Cleaning Composition

Component	Weight %
Alkyl Sulphate	8.0
Alkyl Ethoxy Sulphate	2.0
Mixture of C <sub>25</sub> and C <sub>45</sub> alcohol 3 and 7 times ethoxylated	6.0
Polyhydroxy fatty acid amide	2.5
Zeolite	17.0
Layered silicate/citrate	16.0
Carbonate	7.0
Maleic acid acrylic acid copolymer	5.0
Soil release polymer	0.4
Carboxymethyl cellulose	0.4
Poly (4-vinylpyridine) -N-oxide	0.1
Copolymer of vinylimidazole and vinylpyrrolidone	0.1
PEG2000	0.2
Asn74Asp + Val197Glu + Gln200Glu + Ser210Glu	0.5
Lipase	0.2
Cellulase	0.2
Tetracetylene diamine	6.0
Percarbonate	22.0
Ethylene diamine disuccinic acid	0.3

Suds suppressor	3.5
Disodium-4,4'-bis (2-morpholino -4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulphonate	0.25
Disodium-4,4'-bis (2-sulfostyryl) biphenyl	0.05
Water, Perfume and Minors	Up to 100

## Example 46

## Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulphonate	7.6
C <sub>16</sub> -C <sub>18</sub> alkyl sulfate	1.3
C <sub>14-15</sub> alcohol 7 times ethoxylated	4.0
Coco-alkyl-dimethyl hydroxyethyl ammonium chloride	1.4
Dispersant	0.07
Silicone fluid	0.8
Trisodium citrate	5.0
Zeolite 4A	15.0
Maleic acid acrylic acid copolymer	4.0
Diethylene triamine penta methylene phosphonic acid	0.4
Perborate	15.0
Tetraacetylene diamine	5.0
Smectite clay	10.0
Poly (oxy ethylene) (MW 300,000)	0.3
Tyr208Phe + Leu211Asn	0.4
Lipase	0.2
Amylase	0.3
Cellulase	0.2
Sodium silicate	3.0
Sodium carbonate	10.0
Carboxymethyl cellulose	0.2
Brighteners	0.2
Water, perfume and minors	Up to 100

Example 47  
Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulfonate	6.92
Tallow alkyl sulfate	2.05
C <sub>14-15</sub> alcohol 7 times ethoxylated	4.4
C <sub>12-15</sub> alkyl ethoxy sulfate - 3 times ethoxylated	0.16
Zeolite	20.2
Citrate	5.5
Carbonate	15.4
Silicate	3.0
Maleic acid acrylic acid copolymer	4.0
Carboxymethyl cellulase	0.31
Soil release polymer	0.30
Asn74His + Val197Glu + Pro204Ala + Ala209Thr + Ser210Glu	0.2
Lipase	0.36
Cellulase	0.13
Perborate tetrahydrate	11.64
Perborate monohydrate	8.7
Tetraacetylene diamine	5.0
Diethylene tramine penta methyl phosphonic acid	0.38
Magnesium sulfate	0.40
Brightener	0.19
Perfume, silicone, suds suppressors	0.85
Minors	Up to 100

b. Liquid fabric cleaning compositions

- 5        Liquid fabric cleaning compositions of the present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.005% to about 5%, more preferably from about 0.01% to about 1%, by weight of active enzyme of the composition. Such liquid fabric cleaning compositions typically additionally comprise an anionic surfactant, a
- 10    fatty acid, a water-soluble detergency builder and water.

The liquid fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 48-52  
Liquid Fabric Cleaning Compositions

		Example No.				
Component		48	49	50	51	52
5	Pro204Ala + Ala209Thr	0.05	0.03	0.30	0.03	0.10
	Gln200Glu + Thr207Glu + Ser210Glu	-	-	-	0.01	0.20
	C <sub>12</sub> - C <sub>14</sub> alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
	2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
10	Sodium citrate	1.00	1.00	1.00	1.00	1.00
	C <sub>10</sub> alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
	Monethanolamine	2.50	2.50	2.50	2.50	2.50
	Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

In Examples 48-50 the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Pro204Ala + Ala209Thr, with substantially similar results.

In Examples 51-52, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Pro204Ala + Ala209Thr and Gln200Glu + Thr207Glu + Ser210Glu, with substantially similar results.

Examples 53-57  
Liquid Fabric Cleaning Compositions

		Example No.				
Component		53	54	55	56	57
25	Tyr102Cys + Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln + Thr207Gly	0.05	0.03	0.30	0.03	0.10
	Asn74Asp + Ser97Asp + Gln 57Ser + Asn 60Ser	-	-	-	0.01	0.20
30	C <sub>12</sub> - C <sub>14</sub> alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
	2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
	Sodium citrate	1.00	1.00	1.00	1.00	1.00
	C <sub>10</sub> alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
	Monethanolamine	2.50	2.50	2.50	2.50	2.50
35	Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

In Examples 53-55 the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Tyr102Cys + Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln + Thr207Gly, with substantially similar results.

In Examples 56-57, any combination of the subtilisin 309 variants recited in Tables 212, among others, are substituted for Tyr102Cys + Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln + Thr207Gly and Asn74Asp + Ser97Asp + Gln 57Ser + Asn 60Ser, with substantially similar results.

5

## Examples 58-59

## Granular Fabric Cleaning Composition

	Component	Example No.	
		58	59
	C <sub>12-14</sub> alkenyl succinic acid	3.0	8.0
10	Citric acid monohydrate	10.0	15.0
	Sodium C <sub>12-15</sub> alkyl sulphate	8.0	8.0
	Sodium sulfate of C <sub>12-15</sub> alcohol 2 times ethoxylated	-	3.0
	C <sub>12-15</sub> alcohol 7 times ethoxylated	-	8.0
	C <sub>12-15</sub> alcohol 5 times ethoxylated	8.0	-
15	Diethylene triamine penta (methylene phosphonic acid)	0.2	-
	Oleic acid	1.8	-
	Ethanol	4.0	4.0
	Propanediol	2.0	2.0
	Asn74Asp + Ser210Glu	0.2	0.2
20	Polyvinyl pyrrolidone	1.0	2.0
	Suds suppressor	0.15	0.15
	NaOH	up to pH 7.5	
	Perborate	0.5	1
	Phenol sulphonate	0.1	0.2
25	Peroxidase	0.4	0.1
	Waters and minors	up to 100 parts	

In each of Examples 58 and 59 herein, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Asn74Asp + Ser210Glu, with substantially similar results.

Examples 60-62  
Liquid Fabric Cleaning Composition

		Example No.		
	Component	60	61	62
5	Citric Acid	7.10	3.00	3.00
	Fatty Acid	2.00	-	2.00
	Ethanol	1.93	3.20	3.20
	Boric Acid	2.22	3.50	3.50
	Monoethanolamine	0.71	1.09	1.09
10	1,2 Propanediol	7.89	8.00	8.00
	NaCumene Sulfonate	1.80	3.00	3.00
	NaFormate	0.08	0.08	0.08
	NaOH	6.70	3.80	3.80
	Silicon anti-foam agent	1.16	1.18	1.18
15	Ser210Glu	0.0145	-	-
	Asn74Asp + Ser97Asp + Ser210Glu	-	0.0145	-
	Gln200Glu + Ser210Glu	-	-	0.0145
	Lipase	0.200	0.200	0.200
	Cellulase	-	7.50	7.50
20	Soil release polymer	0.29	0.15	0.15
	Anti-foaming agents	0.06	0.085	0.085
	Brightener 36	0.095	-	-
	Brightener 3	-	0.05	0.05
	C <sub>12</sub> alkyl benzenesulfonic acid	9.86	-	-
25	C <sub>12-15</sub> alkyl polyethoxylate (2.5) sulfate	13.80	18.00	18.00
	C <sub>12</sub> glucose amide	-	5.00	5.00
	C <sub>12-13</sub> alkyl polyethoxylate (9)	2.00	2.00	2.00
	Water, perfume and minors	balance to 100%		

c. Bar fabric cleaning compositions

30 Bar fabric cleaning compositions of the present invention suitable for hand-washing soiled fabrics contain an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about 0.01% to about 1% by weight of the composition.

35 The bar fabric cleaning composition embodiment of the present invention is illustrated by the following examples.



Examples 63-66  
Bar Fabric Cleaning Compositions

		Example No.			
	Component	63	64	65	66
5	Val197Glu	0.3	-	0.1	0.02
	Tyr208Phe + Leu211Asn	-	-	0.4	0.03
	C <sub>12</sub> -C <sub>16</sub> alkyl sulfate, Na	20.0	20.0	20.0	20.00
	C <sub>12</sub> -C <sub>14</sub> N-methyl glucamide	5.0	5.0	5.0	5.00
	C <sub>11</sub> -C <sub>13</sub> alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
10	Sodium carbonate	25.0	25.0	25.0	25.00
	Sodium pyrophosphate	7.0	7.0	7.0	7.00
	Sodium tripolyphosphate	7.0	7.0	7.0	7.00
	Zeolite A (0.1-.10 $\mu$ )	5.0	5.0	5.0	5.00
	Carboxymethylcellulose	0.2	0.2	0.2	0.20
15	Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
	Coconut monethanolamide	5.0	5.0	5.0	5.00
	Brightener, perfume	0.2	0.2	0.2	0.20
	CaSO <sub>4</sub>	1.0	1.0	1.0	1.00
	MgSO <sub>4</sub>	1.0	1.0	1.0	1.00
20	Water	4.0	4.0	4.0	4.00
	Filler*	balance to 100%			

\*Can be selected from convenient materials such as CaCO<sub>3</sub>, talc, clay, silicates, and the like.

In Examples 63-64 the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val197Glu, with substantially similar results.

In Examples 65-66, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val197Glu and Tyr208Phe + Leu211Asn, with substantially similar results.

## Examples 67-70

## Bar Fabric Cleaning Compositions

	Component	Example No.			
		67	68	69	70
5	Asn 60Ser + Val 93Gln + Gly213Asp	0.3	-	0.1	0.02
	Val 93Gln + Tyr102Cys + Thr207Gly + Gly213Asp	-	0.3	0.4	0.03
	C <sub>12</sub> -C <sub>16</sub> alkyl sulfate, Na	20.0	20.0	20.0	20.00
	C <sub>12</sub> -C <sub>14</sub> N-methyl glucamide	5.0	5.0	5.0	5.00
10	C <sub>11</sub> -C <sub>13</sub> alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
	Sodium carbonate	25.0	25.0	25.0	25.00
	Sodium pyrophosphate	7.0	7.0	7.0	7.00
	Sodium tripolyphosphate	7.0	7.0	7.0	7.00
	Zeolite A (0.1-10 $\mu$ )	5.0	5.0	5.0	5.00
15	Carboxymethylcellulose	0.2	0.2	0.2	0.20
	Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
	Coconut monethanolamide	5.0	5.0	5.0	5.00
	Brightener, perfume	0.2	0.2	0.2	0.20
	CaSO <sub>4</sub>	1.0	1.0	1.0	1.00
20	MgSO <sub>4</sub>	1.0	1.0	1.0	1.00
	Water	4.0	4.0	4.0	4.00
	Filler*	balance to 100%			

\*Can be selected from convenient materials such as CaCO<sub>3</sub>, talc, clay, silicates, and the like.

25 In Example 67, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Asn 60Ser + Val 93Gln + Gly213Asp, with substantially similar results.

In Example 68, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val 93Gln + Tyr102Cys + Thr207Gly + Gly213Asp, with substantially similar results.

30 In Examples 69-70, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Asn 60Ser + Val 93Gln + Gly213Asp and Val 93Gln + Tyr102Cys + Thr207Gly + Gly213Asp, with substantially similar results.

#### 35 B. Additional Cleaning Compositions

In addition to the hard surface cleaning, dishwashing and fabric cleaning compositions discussed above, one or more enzyme variants of the present invention may be incorporated into a variety of other cleaning

compositions where hydrolysis of an insoluble substrate is desired. Such additional cleaning compositions include but are not limited to, oral cleaning compositions, denture cleaning compositions, and contact lens cleaning compositions.

5    1.    Oral cleaning compositions

In another embodiment of the present invention, a pharmaceutically-acceptable amount of one or more enzyme variants of the present invention are included in compositions useful for removing proteinaceous stains from teeth or dentures. As used herein, "oral cleaning compositions" refers to  
10   dentifrices, toothpastes, toothgels, toothpowders, mouthwashes, mouth sprays, mouth gels, chewing gums, lozenges, sachets, tablets, biogels, prophylaxis pastes, dental treatment solutions, and the like. Preferably, the oral cleaning compositions comprise from about 0.0001% to about 20% of one or more enzyme variants of the present invention, more preferably from about 0.001%  
15   to about 10%, more preferably still from about 0.01% to about 5%, by weight of the composition, and a pharmaceutically-acceptable carrier. As used herein, "pharmaceutically-acceptable" means that drugs, medicaments or inert ingredients which the term describes are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, incompatibility,  
20   instability, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio.

Typically, the pharmaceutically-acceptable oral cleaning carrier components of the oral cleaning components of the oral cleaning compositions will generally comprise from about 50% to about 99.99%, preferably from  
25   about 65% to about 99.99%, more preferably from about 65% to about 99%, by weight of the composition.

The pharmaceutically-acceptable carrier components and optional components which may be included in the oral cleaning compositions of the present invention are well known to those skilled in the art. A wide variety of  
30   composition types, carrier components and optional components useful in the oral cleaning compositions are disclosed in U.S. Patent 5,096,700, Seibel, issued March 17, 1992; U.S. Patent 5,028,414, Sampathkumar, issued July 2, 1991; and U.S. Patent 5,028,415, Benedict, Bush and Sunberg, issued July 2, 1991; all of which are incorporated herein by reference.

35       The oral cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 71-74  
Dentifrice Composition

		Example No.			
Component		71	72	73	74
5	Val199Leu + Pro204Ala + Thr207Glu +Ser210Glu	2.000	3.500	1.500	2.000
	Sorbitol (70% aqueous solution)	35.000	35.000	35.000	35.000
	PEG-6*	1.000	1.000	1.000	1.000
	Silica dental abrasive**	20.000	20.000	20.000	20.000
10	Sodium fluoride	0.243	0.243	0.243	0.243
	Titanium dioxide	0.500	0.500	0.500	0.500
	Sodium saccharin	0.286	0.286	0.286	0.286
	Sodium alkyl sulfate (27.9% aqueous solution)	4.000	4.000	4.000	4.000
15	Flavor	1.040	1.040	1.040	1.040
	Carboxyvinyl Polymer***	0.300	0.300	0.300	0.300
	Carrageenan****	0.800	0.800	0.800	0.800
	Water	balance to 100%			

\*PEG-6 = Polyethylene glycol having a molecular weight of 600.

20 \*\*Precipitated silica identified as Zeodent 119 offered by J.M. Huber.

\*\*\*Carbopol offered by B.F. Goodrich Chemical Company.

\*\*\*\*Iota Carrageenan offered by Hercules Chemical Company.

In Examples 71-74 the subtilisin 309 variants recited in Tables 3-38,  
among others, are substituted for Val199Leu + Pro204Ala + Thr207Glu +  
25 Ser210Glu, with substantially similar results.

Examples 75-78  
Mouthwash Composition

		Example No.			
	Component	75	76	77	78
5	Ser210Asp	3.00	7.50	1.00	5.00
	SDA 40 Alcohol	8.00	8.00	8.00	8.00
	Flavor	0.08	0.08	0.08	0.08
	Emulsifier	0.08	0.08	0.08	0.08
	Sodium Fluoride	0.05	0.05	0.05	0.05
10	Glycerin	10.00	10.00	10.00	10.00
	Sweetener	0.02	0.02	0.02	0.02
	Benzoic acid	0.05	0.05	0.05	0.05
	Sodium hydroxide	0.20	0.20	0.20	0.20
	Dye	0.04	0.04	0.04	0.04
15	Water	balance to 100%			

In Examples 75-78, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Ser210Asp, with substantially similar results.

Examples 79-82  
Lozenge Composition

		Example No.			
	Component	79	80	81	82
20	Tyr208Phe + Leu211Asn	0.01	0.03	0.10	0.02
	Sorbitol	17.50	17.50	17.50	17.50
	Mannitol	17.50	17.50	17.50	17.50
25	Starch	13.60	13.60	13.60	13.60
	Sweetener	1.20	1.20	1.20	1.20
	Flavor	11.70	11.70	11.70	11.70
	Color	0.10	0.10	0.10	0.10
	Corn Syrup	balance to 100%			

30 In Examples 79-82, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Tyr208Phe + Leu211Asn, with substantially similar results.

Examples 83-86  
Chewing Gum Composition

Component		Example No.			
		83	84	85	86
5	Val199Met + Pro204Ala + Thr207Glu	0.03	0.02	0.10	0.05
	Sorbitol crystals	38.44	38.40	38.40	38.40
	Paloja-T gum base*	20.00	20.00	20.00	20.00
	Sorbitol (70% aqueous solution)	22.00	22.00	22.00	22.00
	Mannitol	10.00	10.00	10.00	10.00
10	Glycerine	7.56	7.56	7.56	7.56
	Flavor	1.00	1.00	1.00	1.00

\*Supplied by L.A. Dreyfus Company.

In Examples 83-86, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val199Met + Pro204Ala + Thr207Glu, with substantially similar results.

## 2. Denture cleaning compositions

In another embodiment of the present invention, denture cleaning compositions for cleaning dentures outside of the oral cavity comprise one or more enzyme variants of the present invention. Such denture cleaning compositions comprise an effective amount of one or more of the enzyme variants, preferably from about 0.0001% to about 50% of one or more of the enzyme variants, more preferably from about 0.001% to about 35%, more preferably still from about 0.01% to about 20%, by weight of the composition, and a denture cleansing carrier. Various denture cleansing composition formats such as effervescent tablets and the like are well known in the art (see for example U.S. Patent 5,055,305, Young, incorporated herein by reference), and are generally appropriate for incorporation of one or more of the enzyme variants for removing proteinaceous stains from dentures.

The denture cleaning composition embodiment of the present invention is illustrated by the following examples.

## Examples 87-90

## Two-layer Effervescent Denture Cleansing Tablet

		Example No.			
Component		87	88	89	90
5	<u>Acidic Layer</u>				
	Ser210Glu	1.0	1.5	0.01	0.05
	Tartaric acid	24.0	24.0	24.00	24.00
	Sodium carbonate	4.0	4.0	4.00	4.00
	Sulphamic acid	10.0	10.0	10.00	10.00
10	PEG 20,000	4.0	4.0	4.00	4.00
	Sodium bicarbonate	24.5	24.5	24.50	24.50
	Potassium persulfate	15.0	15.0	15.00	15.00
	Sodium acid pyrophosphate	7.0	7.0	7.00	7.00
	Pyrogenic silica	2.0	2.0	2.00	2.00
15	TAED*	7.0	7.0	7.00	7.00
	Ricinoleylsulfosuccinate	0.5	0.5	0.50	0.50
	Flavor	1.0	1.0	1.00	1.00
	<u>Alkaline Layer</u>				
	Sodium perborate monohydrate	32.0	32.0	32.00	32.00
20	Sodium bicarbonate	19.0	19.0	19.00	19.00
	EDTA	3.0	3.0	3.00	3.00
	Sodium tripolyphosphate	12.0	12.0	12.00	12.00
	PEG 20,000	2.0	2.0	2.00	2.00
	Potassium persulfate	26.0	26.0	26.00	26.00
25	Sodium carbonate	2.0	2.0	2.00	2.00
	Pyrogenic silica	2.0	2.0	2.00	2.00
	Dye/flavor	2.0	2.0	2.00	2.00

\*Tetraacetylene diamine

30 In Examples 87-90, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Ser210Glu, with substantially similar results.

### 3. Contact Lens Cleaning Compositions

35 In another embodiment of the present invention, contact lens cleaning compositions comprise one or more enzyme variants of the present invention. Such contact lens cleaning compositions comprise an effective amount of one or more of the enzyme variants, preferably from about 0.01% to about 50% of one or more of the enzyme variants, more preferably from about 0.01% to about 20%, more preferably still from about 1% to about 5%, by weight of the composition, and a contact lens cleaning carrier. Various contact lens

cleaning composition formats such as tablets, liquids and the like are well known in the art (see for example U.S. Patent 4,863,627, Davies, Meaken and Rees, issued September 5, 1989; U.S. Patent Re. 32,672, Huth, Lam and Kirai, reissued May 24, 1988; U.S. Patent 4,609,493, Schäfer, issued  
 5 September 2, 1986; U.S. Patent, 4,690,793, Ogunbiyi and Smith, issued September 1, 1987; U.S. Patent 4,614,549, Ogunbiyi, Riedhammer and Smith, issued September 30, 1986; and U.S. Patent 4,285,738, Ogata, issued August 25, 1981; each of which are incorporated herein by reference), and are generally appropriate for incorporation of one or more enzyme variants of the  
 10 present invention for removing proteinaceous stains from contact lens.

The contact lens cleaning composition embodiment of the present invention is illustrated by the following examples.

#### Examples 91-94

##### Enzymatic Contact Lens Cleaning Solution

15	Component	Example No.			
		91	92	93	94
	Val199Leu + Ser210Asp	0.01	0.5	0.1	2.0
	Glucose	50.00	50.0	50.0	50.0
	Nonionic surfactant (polyoxyethylene- 20 polyoxypropylene copolymer)	2.00	2.0	2.0	2.0
	Anionic surfactant (polyoxyethylene- alkylphenylether sodium sulfricester)	1.00	1.0	1.0	1.0
	Sodium chloride	1.00	1.0	1.0	1.0
	Borax	0.30	0.3	0.3	0.3
25	Water	balance to 100%			

In Examples 91-94, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val199Leu + Ser210Asp, with substantially similar results.

While particular embodiments of the subject invention have been  
 30 described, it will be obvious to those skilled in the art that various changes and modifications of the subject invention can be made without departing from the spirit and scope of the invention. It is intended to cover, in the appended claims, all such modifications that are within the scope of the invention.



## SEQUENCE LISTING

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(ii) TITLE OF INVENTION: SUBTILISIN 309 VARIANTS WITH  
DECREASED ADSORPTION AND  
INCREASED HYDROLYSIS

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:  
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(v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:  
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 269 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala  
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp  
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser  
35 40 45

165

5 Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr  
 50 55 60  
 His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu  
 65 70 75 80  
 10 Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala  
 85 90 95  
 15 Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala  
 100 105 110  
 Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser  
 115 120 125  
 20 Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly  
 130 135 140  
 25 Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser  
 145 150 155 160  
 30 Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln  
 165 170 175  
 35 Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile  
 180 185 190  
 40 Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr  
 195 200 205  
 Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala  
 210 215 220  
 45 Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile  
 225 230 235 240  
 50 Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu  
 245 250 255  
 55 Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg  
 260 265

## (2) INFORMATION FOR SEQ ID NO:2:

- 60 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 275 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 65 (ii) MOLECULE TYPE: protein

166

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

5	Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu 1 5 10 15
	His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp 20 25 30
10	Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala 35 40 45
	Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His 50 55 60
15	Gly Thr His Val Ala Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly 65 70 75 80
	Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu 85 90 95
20	Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu 100 105 110
	Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly 115 120 125
25	Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala 130 135 140
	Ser Gly Val Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly 145 150 155 160
30	Ser Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala 165 170 175
35	Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val 180 185 190
	Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr 195 200 205
40	Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser 210 215 220
	Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn 225 230 235 240
45	Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys 245 250 255
50	Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala 260 265 270
55	Ala Ala Gln 275

What is claimed is

1. A subtilisin 309 variant having a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, characterized in that the modified amino acid sequence comprises a substitution at one or more of positions 193, 194, 195, 196, 197, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214, wherein
  - a. when a substitution occurs at position 193, the substituting amino acid is Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
  - b. when a substitution occurs at position 194, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
  - c. when a substitution occurs at position 195, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
  - d. when a substitution occurs at position 196, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
  - e. when a substitution occurs at position 197, the substituting amino acid is Met, Cys, Ala, His, Pro, Gly, Gln, Ser, Asp or Glu;
  - f. when a substitution occurs at position 199, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
  - g. when a substitution occurs at position 200, the substituting amino acid is 200 Asn or Ser;
  - h. when a substitution occurs at position 201, the substituting amino acid is Asp or Glu;
  - i. when a substitution occurs at position 202, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;
  - j. when a substitution occurs at position 203, the substituting amino acid is Ile, Met, Ala, His, Pro, Gln, Asn, Ser, or Asp;
  - k. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
  - l. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
  - m. when a substitution occurs at position 206, the substituting amino acid is Asp or Glu;
  - n. when a substitution occurs at position 207, the substituting amino acid is Pro, Gly, Gln, Asn, Ser or Glu;
  - o. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;

- p. when a substitution occurs at position 209, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- q. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;
- r. when a substitution occurs at position 211, the substituting amino acid is Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, or Asp;
- s. when a substitution occurs at position 212, the substituting amino acid is Glu;
- t. when a substitution occurs at position 213, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and
- u. when a substitution occurs at position 214, the substituting amino acid is Pro, Gly, Gln, Asn, Asp, Ser or Glu;

whereby the subtilisin 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309; preferably

- i. when a substitution occurs at position 200, the substituting amino acid is Ser;
- ii. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
- iii. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;
- iv. when a substitution occurs at position 209, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and
- v. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;

more preferably when a substitution occurs at one or more of positions 193, 194, 195, 196, 197, 199, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, or 214, the substituting amino acid for any of positions 193, 194, 195, 196, 197, 199, 201, 202, 204, 205, 206, 208, 209, 210, 211, 212, 213, or 214 is Asp or Glu; when a substitution occurs at position 203, the substituting amino acid for position 203 is Asp; and when a substitution occurs at one or more of positions 207 or 212, the substituting amino acid for either of positions 207 or 212 is Glu.

2. The subtilisin 309 variant of Claim 1 having a single amino acid substitution wherein the substitution is:

- a. Glu for Thr at position 207,
- b. Glu for Ser at position 210, or
- c. Asp for Ser at position 210.

3. A subtilisin 309 variant having a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, characterized in that the modified amino acid sequence comprises a substitution at three or more of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 is substituted, wherein

- a. when a substitution occurs at position 193, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- b. when a substitution occurs at position 194, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- c. when a substitution occurs at position 195, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
- d. when a substitution occurs at position 196, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
- e. when a substitution occurs at position 197, the substituting amino acid is Met, Cys, Ala, His, Pro, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- f. when a substitution occurs at position 198, the substituting amino acid is Glu, Gln, Asp or Ser;
- g. when a substitution occurs at position 199, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- h. when a substitution occurs at position 200, the substituting amino acid is Asn, Ser Glu, or Asp;
- i. when a substitution occurs at position 201, the substituting amino acid is Asp or Glu;
- j. when a substitution occurs at position 202, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- k. when a substitution occurs at position 203, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- l. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
- m. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;

- n. when a substitution occurs at position 206, the substituting amino acid is Asp or Glu;
- o. when a substitution occurs at position 207, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- p. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Thr, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;
- q. when a substitution occurs at position 209, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- r. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;
- s. when a substitution occurs at position 211, the substituting amino acid is Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Glu, or Asp;
- t. when a substitution occurs at position 212, the substituting amino acid is Gln, Ser, Asp, or Glu;
- u. when a substitution occurs at position 213, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and
- v. when a substitution occurs at position 214, the substituting amino acid is Pro, Gly, Gln, Asn, Asp, Ser or Glu;

whereby the 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309; preferably

- i. when a substitution occurs at position 200, the substituting amino acid is Asn, Glu, Asp, or Ser;
- ii. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
- iii. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
- iv. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;
- v. when a substitution occurs at position 209, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and
- vi. when a substitution occurs at position 210, the substituting amino acid is Glu or Asp.

4. The subtilisin 309 variant of Claim 3 comprising two amino acid substitutions, wherein the substitutions are:

- a. Glu for Thr at position 207 and Glu for Ser at position 210;
- b. Leu for Val at position 197 and Glu for Ser at position 210; or
- c. Leu for Val at position 199 and Asp for Ser at position 210.
- d. Ala for Pro at position 204 and Thr for Ala at position 209;
- e. Phe for Tyr at position 208 and Asn for Leu at position 211;
- f. Asp for Asn at position 74 and Glu for Ser at position 210; or
- g. Glu for Lys at position 207 and Glu for Ala at position 210

5. A subtilisin 309 variant having a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, characterized in that the modified amino acid sequence comprises a substitution at three or more of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 is substituted, wherein

- a. when a substitution occurs at position 193, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- b. when a substitution occurs at position 194, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- c. when a substitution occurs at position 195, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
- d. when a substitution occurs at position 196, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
- e. when a substitution occurs at position 197, the substituting amino acid is Met, Cys, Ala, His, Pro, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- f. when a substitution occurs at position 198, the substituting amino acid is Glu, Gln, Asp or Ser;
- g. when a substitution occurs at position 199, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- h. when a substitution occurs at position 200, the substituting amino acid is Asn, Ser Glu, or Asp;
- i. when a substitution occurs at position 201, the substituting amino acid is Asp or Glu;
- j. when a substitution occurs at position 202, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;



- k. when a substitution occurs at position 203, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
  - l. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
  - m. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
  - n. when a substitution occurs at position 206, the substituting amino acid is Asp or Glu;
  - o. when a substitution occurs at position 207, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;
  - p. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Thr, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;
  - q. when a substitution occurs at position 209, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
  - r. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;
  - s. when a substitution occurs at position 211, the substituting amino acid is Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Glu, or Asp;
  - t. when a substitution occurs at position 212, the substituting amino acid is Gln, Ser, Asp, or Glu;
  - u. when a substitution occurs at position 213, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and
  - v. when a substitution occurs at position 214, the substituting amino acid is Pro, Gly, Gln, Asn, Asp, Ser or Glu;
- whereby the 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309; preferably
- i. when a substitution occurs at position 200, the substituting amino acid is Asn, Glu, Asp, or Ser;
  - ii. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
  - iii. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;

- iv. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;
- v. when a substitution occurs at position 209, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and
- vi. when a substitution occurs at position 210, the substituting amino acid is Glu or Asp .

6. The subtilisin 309 variant of Claim 5 comprising three amino acid substitutions, wherein the substitutions are:

- a. Glu substituted for Gln at position 200, Ala substituted for Gly at position 205, and Glu substituted for Ser at position 210;
- b. Leu substituted for Val at position 199, Ala substituted for Pro at position 204, and Glu substituted for Thr at position 207;
- c. Asp for Asn at position 74, Glu substituted for Gln at position 200, and Glu substituted for Ser at position 210; or
- d. Glu for Gln at position 200, Glu for Thr at position 207, and Glu for Ser at position 210.

7. The subtilisin 309 variant of Claim 5 having the following four substitutions: Ala substituted for Pro at position 204, Glu substituted for Thr at position 207, Glu substituted for Ser at position 210, and Asp substituted for Asn at position 74.

8. The subtilisin 309 variant of Claim 5 having the following five substitutions: Leu for Ile at position 199, Ala for Pro at position 204, Glu for Lys at position 207, Glu for Ala at positions 210, and Asp substituted for Asn at position 74.

9. A subtilisin 309 variant having a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region and a fifth loop region; characterized in that the modified amino acid sequence comprises a substitution at one or more positions in one or more of the loop regions; wherein

- A. when a substitution occurs in the first loop region, the substitution occurs at one or more of positions 57, 58, 59, 60, 61, 63, or 64; wherein

- a. when a substitution occurs at position 57, the substituting amino acid is Asn, Asp, Glu or Ser;
  - b. when a substitution occurs at position 58, the substituting amino acid is Glu;
  - c. when a substitution occurs at position 59, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - d. when a substitution occurs at position 60, the substituting amino acid is Asp, Gln, Glu or Ser;
  - e. when a substitution occurs at position 61, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - f. when a substitution occurs at position 63, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and
  - g. when a substitution occurs at position 64, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser;
- B. when a substitution occurs in the second loop region, the substitution occurs at one or more of positions 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104 or 105; wherein
- a. when a substitution occurs at position 93, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;
  - b. when a substitution occurs at position 94, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val;
  - c. when a substitution occurs at position 95, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - d. when a substitution occurs at position 96, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;
  - e. when a substitution occurs at position 97, the substituting amino acid is Asp or Glu;
  - f. when a substitution occurs at position 98, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - g. when a substitution occurs at position 99, the substituting amino acid is Asp or Glu;
  - h. when a substitution occurs at position 100, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - i. when a substitution occurs at position 101, the substituting amino acid is Asp or Glu;

- j. when a substitution occurs at position 102, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;
  - k. when a substitution occurs at position 103, the substituting amino acid is Asp or Glu;
  - l. when a substitution occurs at position 104, the substituting amino acid is Asp or Glu;
  - m. when a substitution occurs at position 105, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val; and
- C. when a substitution occurs in the third loop region, the substitution occurs at one or more of positions 124, 125, 126, 127, 128, 129, 130 or 131; wherein
- a. when a substitution occurs at position 124, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val;
  - b. when a substitution occurs at position 125, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - c. when a substitution occurs at position 126, the substituting amino acid is Asp or Glu;
  - d. when a substitution occurs at position 127, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;
  - e. when a substitution occurs at position 128, the substituting amino acid is Asp or Glu;
  - f. when a substitution occurs at position 129, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;
  - g. when a substitution occurs at position 130, the substituting amino acid is Asp or Glu; and
  - h. when a substitution occurs at position 131, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser, Thr;
- D. when a substitution occurs in the fourth loop region, the substitution occurs at one or more of positions 152, 153, 154, 155, 156, 157, 158, 159, 160 or 161; wherein
- a. when a substitution occurs at position 152, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - b. when a substitution occurs at position 153, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

- c. when a substitution occurs at position 154, the substituting amino acid is Asp or Glu;
  - d. when a substitution occurs at position 155, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - e. when a substitution occurs at position 156, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;
  - f. when a substitution occurs at position 157, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - g. when a substitution occurs at position 158, the substituting amino acid is Asp or Glu;
  - h. when a substitution occurs at position 159, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val;
  - i. when a substitution occurs at position 160, the substituting amino acid is Asp or Glu; and
  - j. when a substitution occurs at position 161, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val;
- E. when a substitution occurs in the fifth loop region, the substitution occurs at one or more of positions 181, 182, 183, 184 or 185; wherein
- a. when a substitution occurs at position 181, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;
  - b. when a substitution occurs at position 182, the substituting amino acid is Asp or Glu;
  - c. when a substitution occurs at position 183, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr, Tyr or Val;
  - d. when a substitution occurs at position 184, the substituting amino acid is Asp or Glu; and
  - e. when a substitution occurs at position 185, the substituting amino acid is Asn, Asp, Glu or Ser;

whereby the subtilisin 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309.

10. The subtilisin 309 variant of Claim 9, wherein the subtilisin 309 wild-type amino acid sequence further comprises a sixth loop region, wherein the modified amino acid sequence further comprises one or more substitutions

in the sixth loop region; wherein the substitution(s) in the sixth loop region occurs at one or more of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214; wherein

- a. when a substitution occurs at position 193, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;
- b. when a substitution occurs at position 194, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;
- c. when a substitution occurs at position 195, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;
- d. when a substitution occurs at position 196, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
- e. when a substitution occurs at position 197, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;
- f. when a substitution occurs at position 198, the substituting amino acid is Asp, Gln, Glu or Ser;
- g. when a substitution occurs at position 199, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;
- h. when a substitution occurs at position 200, the substituting amino acid is Asn, Asp, Glu or Ser;
- i. when a substitution occurs at position 201, the substituting amino acid is Asp or Glu;
- j. when a substitution occurs at position 202, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser;
- k. when a substitution occurs at position 203, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val;
- l. when a substitution occurs at position 204, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;
- m. when a substitution occurs at position 205, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
- n. when a substitution occurs at position 206, the substituting amino acid is Asp or Glu;
- o. when a substitution occurs at position 207, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser;

- p. when a substitution occurs at position 208, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val;
- q. when a substitution occurs at position 209, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;
- r. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;
- s. when a substitution occurs at position 211, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val;
- t. when a substitution occurs at position 212, the substituting amino acid is Asp, Gln, Glu or Ser;
- u. when a substitution occurs at position 213, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and
- v. when a substitution occurs at position 214, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

11. A cleaning composition selected from the group consisting of a hard surface cleaning composition, a dishwashing composition, an oral cleaning composition, a denture cleansing composition, a contact lens cleaning composition and a fabric cleaning composition, characterized in that the cleaning composition comprises the subtilisin 309 variant of any of Claims 1-11 and a cleaning composition carrier.

12. The subtilisin 309 variant of any of Claims 1-11 wherein the modified amino acid sequence further comprises His or Asp substituted for Asn at position 74.

13. The cleaning composition of Claim 11, wherein the cleaning composition is a hard surface cleaning composition.

14. The cleaning composition of Claim 11; wherein the cleaning composition is a fabric cleaning composition; preferably the composition is in the form of a liquid.

15. A DNA sequence 309 gene encoding the subtilisin 309 variant of any of Claims 1-11.

**B. amyloliquefaciens**

**B. lentus**

01 10 20 30  
A Q S V P Y G V S Q I K A P A L H S Q G Y T G S N V K V A V I D S G I D S S H P  
A Q S V P W G I S R V Q A P A A H N R G L T G S G V K V A V L D T G I S T \* H P  
41 50 60 70  
D L K V A G C A S M V P S E T N P F Q D N N S H G T H V A G T V A A L N N S I G  
D L N I R G G A S F V P G E \* P S T Q D G N G H G T H V A G T I A A L N N S I G  
81 90 100 110  
V L G V A P S A S L Y A V K V L G A D G S G Q Y S W I I N G I E W A I A N N M D  
V L G V A P S A E L Y A V K V L G A S G S G S V S S I A Q G L E W A C N N G M H  
121 130 140 150  
V I N M S L G G P S G S A A L K A A V D K A V A S G V V V A A A G N E G T S G  
V A N L S L G S P S P S A T L E Q A V N S A T S R G V L V V A A S G N S G A G S

**FIG 1/A**



161 170 180 190  
S S S T V G Y P G K Y P S V I A V G A V D S S N Q R A S F S S V G P E L D V M A  
\* \* \* I S Y P A R Y A N A M A V G A T D Q N N N R A S F S Q Y G A G L D I V A  
201 210 220 230  
P G V S I Q S T L P G N K Y G A Y N G T S M A S P H V A G A A A L I L S K H P N  
P G V N V Q S T Y P G S T Y A S L N G T S M A T P H V A G A A A L V K Q K N P S  
241 250 260 270  
W T N T Q V R S S L E N T T T K L G D S F Y Y G K G L I N V Q A A A Q  
W S N V Q I R N H L K N T A T S L G S T N L Y G S G L V N A E A A T R

FIG 1/B